Echogenicity, pregnancy and the pelvic floor, an observational ultrasound study

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Echogenicity, pregnancy and the pelvic floor, an observational ultrasound study

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een observationeel echo onderzoek
(met een samenvatting in het Nederlands)

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Maria Kristina Verheijen - van de Waarsenburg
geboren op 9 november 1985 te Helmond

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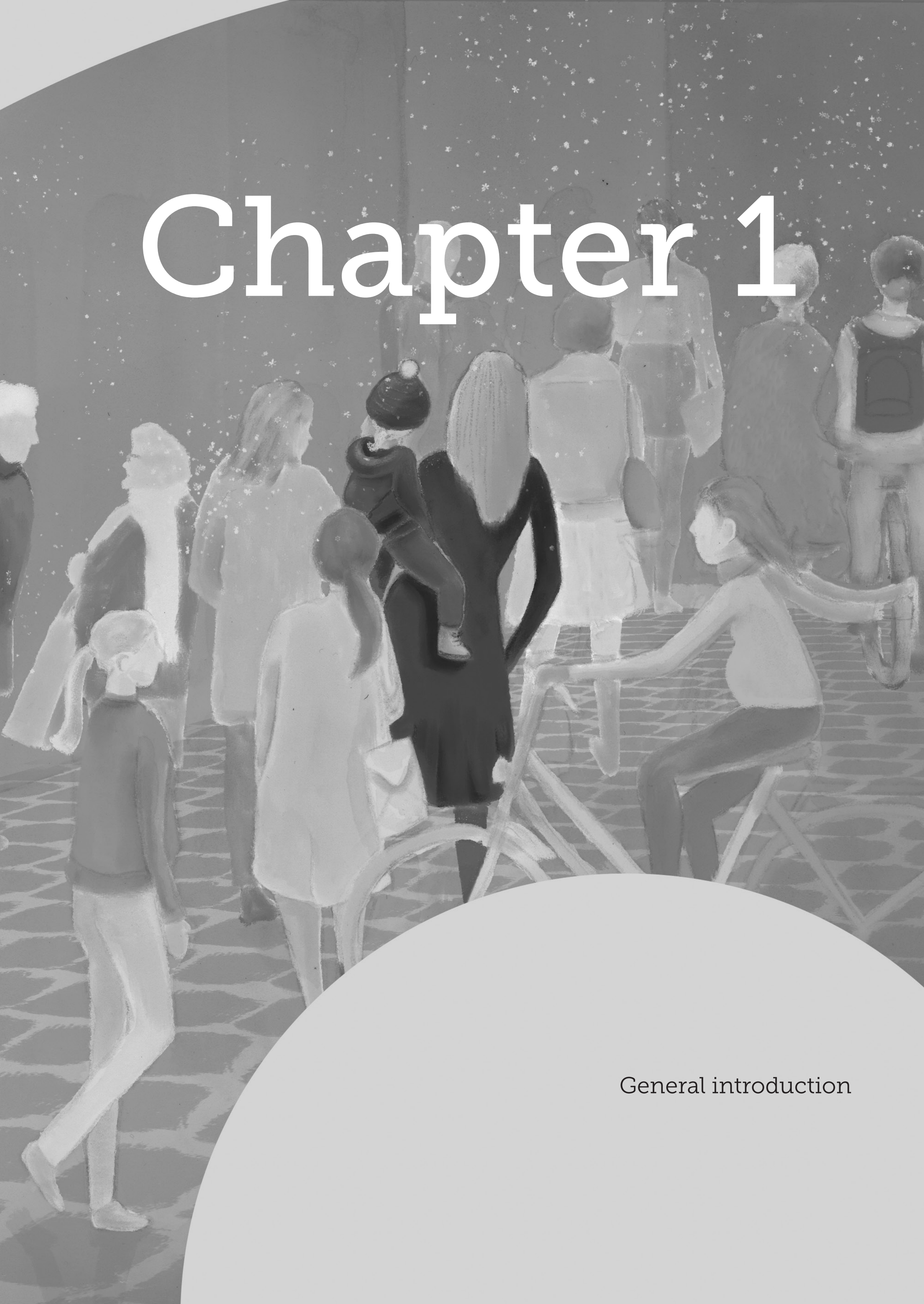
Copromotor:	Dr. M.I.J. Withagen

Contents

Chapter 1



General introduction

General introduction

Anatomy of the pelvic floor

The pelvic floor consists of muscles and connective tissue. The most important muscle of the pelvic floor is the levator ani muscle complex which consists of three parts: the pubococcygeus, the puborectalis and the iliococcygeus muscle. These muscles enclose the genital hiatus, through which the urethra, vagina and rectum pass. The pubococcygeus and puborectalis muscle originate from the posterior left and posterior right side of the pubic symphysis, are U-shaped and loop posteriorly behind the rectum. The iliococcygeus muscle is more horizontally oriented and forms the base on which the pelvic organs rest.

The iliococcygeus muscle acts like a hammock providing passive support to the pelvic organs and lifts the pelvic organs when contracted. The puborectalis muscle is involved in active narrowing and ventral displacement of the genital hiatus, thereby closing the bladder neck, vagina and rectum. The support of the pelvic floor to the pelvic organs is described by Delancey in three levels of support.[1] The apical attachment of the uterus to the sacrum is provided by the cardinal-uterosacral ligament complex; the connective tissue of the middle part of the vagina is bilaterally attached to the arcus tendineus fascia pelvis and the fascia overlying the levator ani muscle. The lower part of the vagina is supported by the urogenital diaphragm and the perineal body.[2]

Pregnancy and the pelvic floor

During vaginal delivery, the pelvic floor muscles are stretched due to the passage of the fetal head. To gain more knowledge about these forces during the delivery, Sindhwani *et al.* used three dimensional magnetic resonance image sequences from a live childbirth to reconstruct the levator ani muscles and fetal head to determine the stretch ratio during vaginal delivery.[2] They found that the pelvic floor muscles are stretched with a ratio of 2.5 (248%).[2] This stretch could lead to (micro) hematoma, edema and abruption of the levator ani from the pubic symphysis (levator ani muscle avulsion). Therefore, childbirth is an important risk factor for pelvic floor dysfunction, with urinary incontinence, fecal incontinence and pelvic organ prolapse as its clinical presentations.[3,4] These pelvic floor disorders are highly prevalent and have a negative impact on the quality of life.[5-8] However, symptoms of incontinence or pelvic organ prolapse may take years to develop. The current concept is that damage to the levator ani muscle leads to a disbalance between intra- and extra abdominal pressure, putting strain on the endopelvic fascia and ligaments that provide support to the pelvic organs. Over time, these fascia structures may fail under continuous strain, with prolapse, urinary and defecatory symptoms as a consequence. In order to develop new ways of treating patients with urogenital dysfunction it is crucial to better understand the alterations of the levator ani muscle complex during pregnancy and after (vaginal) delivery. Transperineal ultrasound is currently considered as the cornerstone of pelvic floor imaging. It is easy accessible, causes minimal discomfort and is relatively inexpensive.

Ultrasound of the pelvic floor

By the use of two-dimensional (2D) transperineal ultrasound, it is possible to visualize the pelvic floor and the pelvic organs (Figure 1). The ultrasound images can be obtained with the pelvic floor at rest, on maximum pelvic floor muscle contraction and during maximum Valsalva maneuver. The visualization of the puborectalis muscle is impossible within 2D, but three-dimensional (3D) transperineal ultrasound permits reliable assessment of hiatal dimensions, echogenicity and area of the puborectalis muscle (Figure 2).[9,10]

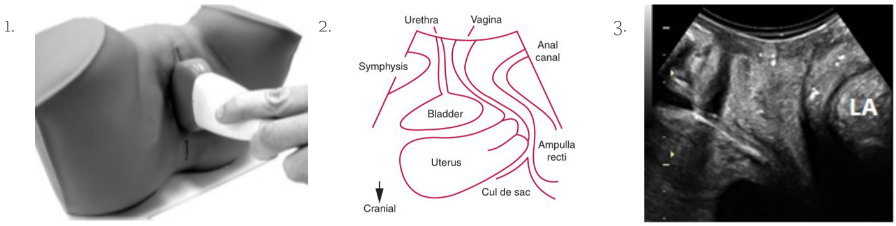


Figure 1. 1. Transperineal ultrasound examination with the women in supine position. 2. Two-dimensional view of the mid-sagittal plane of the pelvic floor with the symphysis, urethra, bladder, vagina, uterus and anal canal. 3. LA=the levator ani muscle passing behind the rectum.

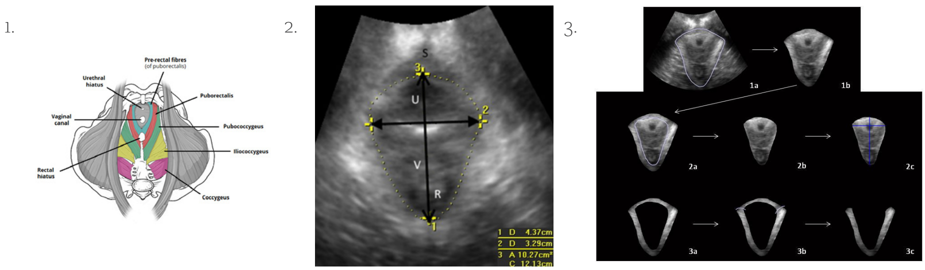


Figure 2. 1. Anatomy of the pelvic floor. 2. Three-dimensional transperineal ultrasound examination in the axial plane with manual measurements of levator hiatal dimensions. Arrow represents anteroposterior (AP) diameter of levator hiatus (1); filled arrow represents transverse diameter of levator hiatus (2); dotted line represents area of levator hiatus (3); S=symphysis pubis; U=urethra; V=vagina; R=rectum. 3. Delineation method of the puborectalis muscle to obtain the area and echogenicity of the puborectalis muscle and the hiatal dimensions.[10]

In the last decade the work of Dietz *et al.* has greatly improved our understanding of the pelvic floor anatomy.[11] Post hoc analyses of 3D ultrasound images made it possible to view the levator ani complex in the axial plane. This in turn allows assessment of hiatal dimensions and area and structure of the puborectalis muscle. The AP diameter of the levator hiatus is defined as the distance from the inferior border of the symphysis pubis to the inner border of the puborectalis muscle. The transverse diameter of the levator hiatus was defined as the longest distance between two inner sides of the puborectalis muscle, perpendicular to the AP diameter. The area of the levator hiatus is measured as the area bordered by the puborectalis muscle, the symphysis pubis and the inferior pubic ramus (Figure 2-2 and 2-3). The structure of a muscle can be assessed with the use of grey-scale analyses of the pixels of the image. Echogenicity of a muscle represents the ratio of muscle fibers and extracellular matrix (ECM). Muscle cells have a low echogenicity and appear dark on the ultrasound image and ECM has a high echogenicity and appears bright on the ultrasound image. Echogenicity is a greyscale which ranges from 0 to 255 without a unit. In previous studies we have shown that the echogenicity of the puborectalis muscle changes during pregnancy and after delivery.[12]

Ultrasound studies of the levator ani muscle during pregnancy and after delivery

Several previous studies used transperineal ultrasound to assess changes in pelvic floor anatomy during pregnancy and after delivery.

Pregnancy

One of the most important pelvic floor disorders is stress urinary incontinence (SUI). During pregnancy, up to 15.4% develop SUI, and at 12 months after delivery 10.5% still report SUI.[13] Therefore pregnancy and delivery play an important role in the pathophysiology of SUI. Ultrasound studies that addressed the issue of SUI and pregnancy focused on the mobility of the bladder neck as marker of urethral support. They showed a more caudal and dorsal position of the bladder neck (increase in urethral mobility) on Valsalva in women with SUI after childbirth.[1,14-17] However, normal urethral function depends not only on the support of the urethra by the peri-urethral ligaments, but also on the intrinsic closure mechanism of the urethral musculature. One of the aims of our study was to assess urethral sphincter echogenicity during and after pregnancy and its association with SUI symptoms. Furthermore, SUI during pregnancy is also associated with an increased hiatal area of the levator.[18] The puborectalis muscle encloses the hiatal area and therefore the contraction ability of the puborectalis muscle is directly related to the size of the hiatal area. Therefore, an increased diameter of the levator hiatus in women with SUI may be caused by structural changes (MEP and/or PMA) of the puborectalis muscle. If the puborectalis muscle contracts less well, we expect a higher echogenicity due to an increase in ECM or decrease in muscle cell quantity. Therefore we wanted to assess the association between SUI symptoms and the structural changes in the puborectalis muscle by the MEP and PMA during first pregnancy.

Mode of delivery

Previously we studied the possible association between ultrasound parameters of the pelvic floor during pregnancy and mode of delivery. Van Veelen *et al*. reported that women who delivered by cesarean section due to failure to progress had a significantly smaller transverse diameter of the levator ani hiatus at 12 weeks' pregnancy during contraction compared to women who had a spontaneous vaginal delivery, and a trend towards a smaller hiatal area in women who delivered by cesarean section due to failure to progress.[19] The structure of the puborectalis muscle, expressed as MEP, in relation to the mode of delivery was studied by Grob *et al*. in the same population.[20] In normal pregnancy, an increase of the collagen content of the intramuscular extracellular matrix and fat mass is associated with a higher muscular echogenicity.[21,22] However, we previously demonstrated that women who delivered by cesarean section due to failure to progress had a significantly lower mean echogenicity of the puborectalis muscle during contraction at 12 weeks' pregnancy as compared to women who have had a vaginal delivery.[20] Therefore the lower echogenicity in women with a caesarean section due to failure to progress may be an indication of a disturbed adaptation of the collagen mechanism and may have profound implications for management. It has to be noted however that the number of women with a caesarean section due to failure to progress in this single center study was small and the difference in MEP was only observed during contraction. Therefore, we set out to study this finding by Grob *et al*. in another independent sample of nulliparous women.[20] Examination in pregnant rats showed an increase in the ECM of the pelvic floor muscles during pregnancy as well, but no differences in the ECM of the tibialis anterior muscle.[21,23] This interesting finding suggests that pregnancy hormones only affect the pelvic floor muscles of pregnant rats. In order to make clear if the increased collagen during pregnancy is local or systemical, we investigated the vastus lateralis muscle that had no relation with the pelvic floor muscles, just like the tibialis muscle in rats. Thereby we assume that a lower echogenicity of the puborectalis muscle alone, is not the main reason for failure to progress, because most of the women were not able to reach full dilation. We hypothesize that a lower echogenicity of the cervix is found as a resultant of less collagen attended with lesser weakening of the cervix.

After delivery

As described before, the levator ani muscle is stretched to at least twice its initial length during passage of the fetal head.[24] Trauma during delivery is known as one of the most important causes of pelvic floor disorders. Levator avulsions, hematoma and a large hiatal area are associated with prolapse symptoms and stress urinary incontinence.[16,18,25,26] Not all vaginally parous women develop pelvic floor disorders and it is still not fully understood who will experience symptoms. The extent of the damage that occurs during delivery and/or differences in the recovery process may be related to this development. Little is known about normal recovery of the pelvic floor in the first weeks after vaginal delivery. In particular, there is a paucity of longitudinal data on this recovery. A better understanding of the recovery of the pelvic floor after vaginal delivery is of importance when considering early interventions to improve this recovery by early interventions with for example stem cells and its culture medium.[27-29]

Outline of this thesis

In chapter 2 we set out to develop a reliable method to measure the area and mean echogenicity of the mid-urethra using 3/4D transperineal ultrasound, as a representation of the urethral sphincter, during pregnancy and after delivery and assess their changes over time.

In chapter 3 we set out to assess the association between the puborectalis muscle area and mean echogenicity and stress urinary incontinence symptoms in women during and after their first ongoing pregnancy, using 3/4D transperineal ultrasound.

In chapter 4 we investigate whether structural changes of the puborectalis muscle during pregnancy are associated with the mode of delivery in their first ongoing pregnancy. And since we hypothesize that echogenicity is an expression of structural change in not only the pelvic floor, we also investigate the cervix and vastus lateralis muscle.

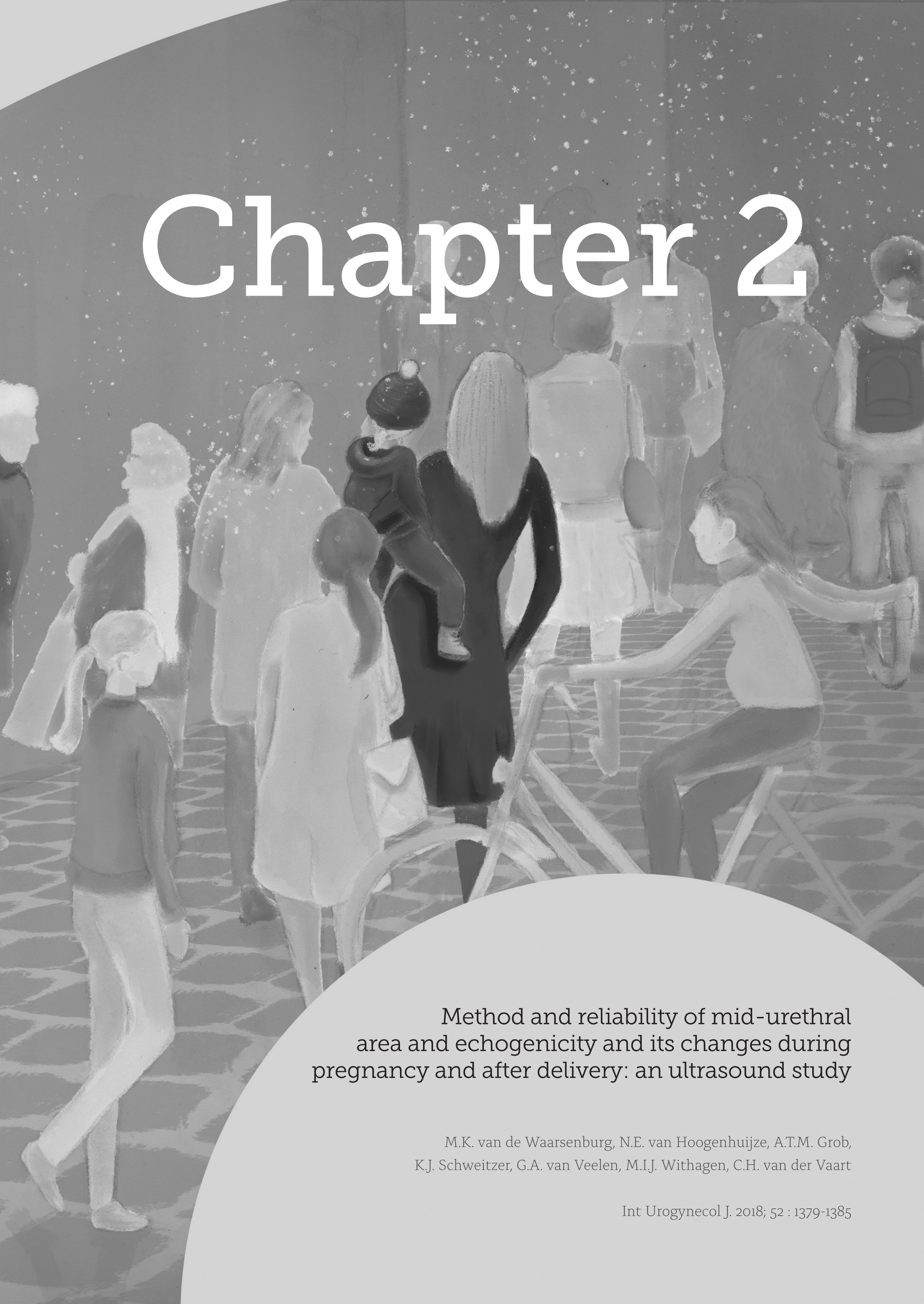
In chapter 5 we investigate the hiatal dimensions using 3/4D transperineal ultrasound by repeated measurements after vaginal delivery and compare them with early pregnancy state to provide more insight in "normal" pelvic floor recovery after first vaginal delivery .

In chapter 6 we evaluate the structural composition of the puborectalis muscle using 3/4D transperineal ultrasound at several moments in time to provide more insight in the recovery after vaginal delivery by the mean echogenicity and puborectalis area measurements.

References

1. DeLancey JO, Miller JM, Kearney R, Howard D, Reddy P, Umek W et al. Vaginal birth and de novo stress incontinence: Relative contributions of urethral dysfunction and mobility. *Obstet Gynecol* 2007; 110 : 354-362.
2. Sindhwani N, Bamberg C, Famaey N, Callewaert G, Dudenhausen JW, Teichgraber U et al. In vivo evidence of significant levator ani muscle stretch on MR images of a live childbirth. *Am J Obstet Gynecol* 2017; 217 : 194.e1,194.e8.
3. MacLennan AH, Taylor AW, Wilson DH, Wilson D. The prevalence of pelvic floor disorders and their relationship to gender, age, parity and mode of delivery. *Bjog* 2000; 107 : 1460-1470.
4. Rortveit G, Hannestad YS, Daltveit AK, Hunskaar S. Age- and type-dependent effects of parity on urinary incontinence: The norwegian EPINCONT study. *Obstet Gynecol* 2001; 98 : 1004-1010.
5. Jelovsek JE, Maher C, Barber MD. Pelvic organ prolapse. *Lancet* 2007; 369 : 1027-1038.
6. Dannecker C and Anthuber C. The effects of childbirth on the pelvic-floor. *J Perinat Med* 2000; 28 : 175-184.
7. Gyhagen M and Milsom I. Prevalence of urinary incontinence (UI) 20 years after childbirth in a national cohort study in singleton primiparae after vaginal or caesarean delivery--authors' reply. *Bjog* 2013; 120 : 1152-1153.
8. Skinner EM and Dietz HP. Psychological and somatic sequelae of traumatic vaginal delivery: A literature review. *Aust N Z J Obstet Gynaecol* 2015; 55 : 309-314.
9. Van Veelen GA, Schweitzer KJ, van der Vaart CH. Reliability of pelvic floor measurements on three- and four-dimensional ultrasound during and after first pregnancy: Implications for training. *Ultrasound Obstet Gynecol* 2013; 42 : 590-595.
10. Grob AT, Veen AA, Schweitzer KJ, Withagen MI, van Veelen GA, van der Vaart CH. Measuring echogenicity and area of the puborectalis muscle: Method and reliability. *Ultrasound Obstet Gynecol* 2014; 44 : 481-485.
11. Dietz HP, Shek C, Clarke B. Biometry of the pubovisceral muscle and levator hiatus by three-dimensional pelvic floor ultrasound. *Ultrasound Obstet Gynecol* 2005; 25 : 580-585.
12. Grob AT, Withagen MI, van de Waarsenburg MK, Schweitzer KJ, van der Vaart CH. Changes in the mean echogenicity and area of the puborectalis muscle during pregnancy and postpartum. *Int Urogynecol J* 2016; 27 : 895-901.
13. Van Brummen HJ, Bruinse HW, van de Pol G, Heintz AP, van der Vaart CH. Bothersome lower urinary tract symptoms 1 year after first delivery: Prevalence and the effect of childbirth. *BJU Int* 2006; 98 : 89-95.
14. Shek KL, Dietz HP, Kirby A. The effect of childbirth on urethral mobility: A prospective observational study. *J Urol* 2010; 184 : 629-634.
15. Chan SS, Cheung RY, Yiu KW, Lee LL, Leung TY, Chung TK. Pelvic floor biometry during a first singleton pregnancy and the relationship with symptoms of pelvic floor disorders: A prospective observational study. *Bjog* 2014; 121 : 121-9.
16. Falkert A, Willmann A, Endress E, Meint P, Seelbach-Gobel B. Three-dimensional ultrasound of pelvic floor: Is there a correlation with delivery mode and persisting pelvic floor disorders 18-24 months after first delivery? *Ultrasound Obstet Gynecol* 2013; 41 : 204-209.
17. Jundt K, Scheer I, Schiessl B, Karl K, Friese K, Peschers UM. Incontinence, bladder neck mobility, and sphincter ruptures in primiparous women. *Eur J Med Res* 2010; 15 : 246-252.

18. Van Veelen A, Schweitzer K, van der Vaart H. Ultrasound assessment of urethral support in women with stress urinary incontinence during and after first pregnancy. *Obstet Gynecol* 2014; 124 : 249-256.
19. Van Veelen GA, Schweitzer KJ, van Hoogenhuijze NE, van der Vaart CH. Association between levator hiatal dimensions on ultrasound during first pregnancy and mode of delivery. *Ultrasound Obstet Gynecol* 2015; 45 : 333-338.
20. Grob AT, Withagen MI, van de Waarsenburg MK, Schweitzer KJ, van der Vaart CH. Association of first-trimester echogenicity of the puborectalis muscle with mode of delivery. *Obstet Gynecol* 2016; 127 : 1021-1026.
21. Alperin M, Kaddis T, Pichika R, Esparza MC, Lieber RL. Pregnancy-induced adaptations in intramuscular extracellular matrix of rat pelvic floor muscles. *Am J Obstet Gynecol* 2016; 215 : 210.e1,210.e7.
22. Abeysekera MV, Morris JA, Davis GK, O'Sullivan AJ. Alterations in energy homeostasis to favour adipose tissue gain: A longitudinal study in healthy pregnant women. *Aust N Z J Obstet Gynaecol* 2016; 56 : 42-48.
23. Alperin M, Lawley DM, Esparza MC, Lieber RL. Pregnancy-induced adaptations in the intrinsic structure of rat pelvic floor muscles. *Am J Obstet Gynecol* 2015; 213 : 191.e1,191.e7.
24. Lien KC, Mooney B, DeLancey JO, Ashton-Miller JA. Levator ani muscle stretch induced by simulated vaginal birth. *Obstet Gynecol* 2004; 103 : 31-40.
25. Chan SS, Cheung RY, Yiu KW, Lee LL, Chung TK. Prevalence of urinary and fecal incontinence in chinese women during and after their first pregnancy. *Int Urogynecol J* 2013; 24 : 1473-1479.
26. Van Delft K, Thakar R, Shobeiri SA, Sultan AH. Levator hematoma at the attachment zone as an early marker for levator ani muscle avulsion. *Ultrasound Obstet Gynecol* 2014; 43 : 210-217.
27. Callewaert G, Albersen M, Janssen K, Damaser MS, Van Mieghem T, van der Vaart CH et al. The impact of vaginal delivery on pelvic floor function - delivery as a time point for secondary prevention. *Bjog* 2016; 123 : 678-681.
28. Callewaert G, Da Cunha MMCM, Sindhwani N, Sampaolesi M, Albersen M, Deprest J. Cell-based secondary prevention of childbirth-induced pelvic floor trauma. *Nat Rev Urol* 2017; 14 : 373-385.
29. Deng K, Lin DL, Hanzlicek B, Balog B, Penn MS, Kiedrowski MJ et al. Mesenchymal stem cells and their secretome partially restore nerve and urethral function in a dual muscle and nerve injury stress urinary incontinence model. *Am J Physiol Renal Physiol* 2015; 308 : F92-F100.



Chapter 2

Method and reliability of mid-urethral area and echogenicity and its changes during pregnancy and after delivery: an ultrasound study

M.K. van de Waarsenburg, N.E. van Hoogenhuijze, A.T.M. Grob, K.J. Schweitzer, G.A. van Veelen, M.I.J. Withagen, C.H. van der Vaart

Int Urogynecol J. 2018; 52 : 1379-1385

Abstract

Objective: To develop a reliable method to measure the area and mean echogenicity of the mid-urethra during and after pregnancy and to assess changes over time.

Methods: For the reliability study, 3D/4D transperineal ultrasound images of 40 pregnant nulliparous women were used. Two observers independently segmented the urethra as follows: in the sagittal plane the urethra was positioned vertically, the marker was placed in the middle section of the lumen of the urethra and eight tomographic ultrasound images of 2.5mm slices were obtained. The central image of the urethra was selected and area and mean echogenicity were calculated automatically. Intra- and interobserver reliability were determined by intraclass correlation coefficients (ICC) and their 95%-CI. Twohundredeighty women underwent transperineal ultrasound at 12 weeks and 36 weeks pregnancy and 6 months postpartum. Paired t-tests were used to assess changes in echogenicity and area.

Results: The ICC for measuring the area was substantial, 0.77; the ICC for measuring mean echogenicity was almost perfect, 0.86. In the total study group (n= 280), the mid-urethral area and mean echogenicity were significantly lower at 6 months after delivery compared to area and echogenicity at 12 and 36 weeks of gestation.

Conclusion: Our protocol for measuring area and mean echogenicity of the mid-urethra is reliable. This study indicates that structural changes in the mid-urethra, in relationship to pregnancy, occur.

Introduction

Stress urinary incontinence (SUI) is a well-known clinical problem and pregnancy and childbirth are important risk factors for the development of SUI.[1,2] Maintaining continence is dependent on normal bladder activity and an intact urethral function. The function of the urethra depends on two mechanism: First, urethral support is provided by the peri-urethral fascia and the endopelvic ligaments, which allows the urethra to close during intra-abdominal pressure rise; the second mechanism is the internal closure mechanism of the urethral sphincter itself.[3]

Transperineal ultrasound can be used to assess urethral support by measuring the urethral and bladder neck mobility during pregnancy and after delivery and is well-investigated before.[4-11]

In contrast to studies on bladder neck and urethral mobility, little is known about possible changes of the urethral sphincter muscle during pregnancy. The urethral sphincter function depends on muscle quantity (volume/thickness),[12,13] muscle composition (proportion of muscle fibers and extracellular matrix (ECM)) and innervation.[14,15]

The muscle quantity of the urethral sphincter has been studied before. A smaller volume/thickness of the urethral sphincter, measured by transvaginal ultrasound, was shown to correlate with SUI.[16,17]

Structural composition of muscle tissue can be assessed indirectly by measuring echogenicity (greyscale with a range 0-255).[18,19] Echogenicity reflects the ratio between muscle cells (dark on ultrasound) and extracellular matrix (ECM). In general, a low echogenicity reflects a predominance of muscle fibers, and a high echogenicity reflects more ECM.[18] Mitterberger *et al.* investigated the urethra with a transurethral ultrasound.[20] Lesions, presenting as hyperechoic structures, were seen more frequently in the urethral sphincter of incontinent women than in the urethral sphincter of continent women.[20]

We designed this study to develop a reliable method to measure the area and mean echogenicity of the mid-urethra, as a representation of the urethral sphincter, during and after pregnancy with 3D/4D transperineal ultrasound, and to assess its changes over time.

Methods

This study is a sub-analysis of a prospective observational study on the association between urogenital symptoms and pelvic floor anatomy during and after pregnancy.[6] Two hundred eighty nulliparous women with a singleton pregnancy and good knowledge of the Dutch language were recruited. All participants underwent 3D/4D transperineal ultrasound assessment at 12 weeks' and 36 weeks' gestation, and 6 months after delivery. Volume imaging datasets were obtained at rest, on maximum pelvic floor muscle contraction and on maximum Valsalva maneuver. The women had an empty bladder. Exclusion criteria were a medical history with urinary or faecal incontinence, anti-incontinence or prolapse surgery, neurological disorders or connective tissue diseases, and an inability to perform maximal Valsalva maneuver due to pulmonary or heart disease. This study has been approved by the institutional Human Research Ethics Committee (reference 08-299); all women gave written informed consent.

For the reliability study, a random selection of ultrasound images at 12 weeks' gestation in 40 participants with an uncomplicated pregnancy and vaginal delivery was used. A GE Voluson 730 Expert ultrasound system with a RAB 4-8MHz curved array volume transducer (GE Healthcare, Hoevelaken, The Netherlands) was used, and settings that could influence echogenicity were set at constant values, as described by Scholten *et al*.[21] The used settings are: gain 15, power 100, Harmonics mid, contrast 8, grey map 4, persistence 8, and enhance 3.

Offline analysis was performed using 4D View 7.0 (GE Medical Systems Kretztechnik, Zipf, Austria) and Matlab® R2010a (MathWorks, Natick, MA). Image analysis in 4D View was performed by determining a point of time at rest and at maximal pelvic floor muscle contraction. As shown in figure 1: In the sagittal plane, the urethra was positioned vertically by rotation around its axis (1), after which tomographic ultrasound images (TUI) with 2.5mm slices were made in the transverse plane at approximately mid-urethral position (2). From these TUI-slices, the observer selected the image with optimal urethra visualization (2). With the use of Matlab® software (imellipse function) the inner and outer border of the mid-urethra were delineated (3, 4). In the remaining ring, consisting of urethral striated muscles, the urethral smooth muscle, and the submucosa, containing vascular elements, the area of the urethral sphincter (cm^2) and mean echogenicity (based on a greyscale image with a range from 0 (black) to 255 (white)) were calculated automatically (5).

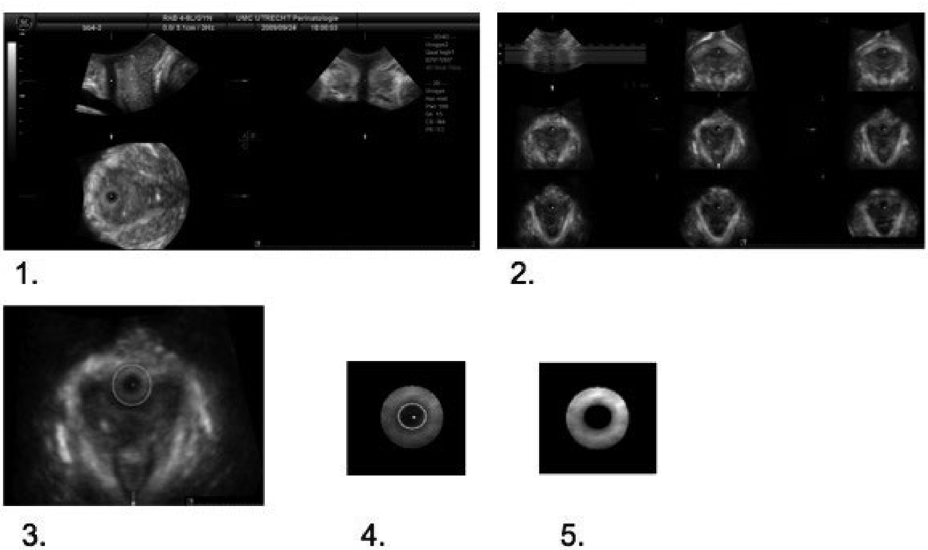


Figure 1. Delineation of the middle part of the urethra.
1. The urethra in vertical position, upper left in the sagittal plane: left, ventral; down, dorsal; right, caudal; up, cranial. 2. Overview of 8 TUI slices at 2.5mm distance. The middle image is selected as the optimal image. 3. Selection of the urethra in the transverse plane, TUI of the urethra with the selection of the outer border of the urethra. 4. Selection of the urethral lumen. 5. Final image of the region of interest (ROI).

Reliability testing, within as well as between observers, was performed on the 40 datasets both at rest and on contraction. Intraobserver reliability was performed in two separate randomized orders to prevent recall bias. The second observer was instructed according to the protocol described, and performed blinded measurements of all 40 datasets once.

Statistical analysis was performed using SPSS (21.0, 2012, Chicago, IL, USA). Intraclass correlation coefficients (ICCs) with 95%-confidence intervals (95%-CI) were calculated and interpreted using classification according to Landis and Koch (0.00-0.20 slight, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.80 substantial, and 0.81-1.00 almost perfect concordance).[22] Mean difference and limits of agreement (LOA) were determined according to the Bland-Altman method.[23]

The changes of mid-urethral area and mean echogenicity over time were assessed with paired-samples t tests. A p-value <0.05 was considered statistical significant.

Results

Intraobserver reliability was substantial for measurements of mid-urethral area (ICC 0.77) and almost perfect for mid-urethral echogenicity (ICC 0.86) (Table 1). Inter-observer reliability was moderate for mid-urethral area (ICC 0.53) and almost perfect for mid-urethral echogenicity (ICC 0.82) (Table 1). Bland-Altman plots of inter-observer reliability for mid-urethral area and mid-urethral echogenicity are shown in figure 2 and 3, respectively.

Table 1. Intra- and interobserver reliability (n=40)

	Intra-observer reliability				Interobserver reliability			
	Mean	(SD)	ICC	(95%-CI)	ICC	(95%-CI)	MD	LOA
Area, cm^2								
Rest	1.68	(0.52)	0.67	(0.23 – 0.70)	0.59	(0.22 – 0.79)	-0.01	-1.02 – 0.99
PFMC	1.57	(0.58)	0.84	(0.68 – 0.92)	0.46	(-0.03 – 0.72)	0.02	-0.61 – 1.26
Overall*	1.63	(0.55)	0.77	(0.63 – 0.85)	0.53	(0.26 – 0.70)	0.00	-1.12 – 1.13
Mean echogenicity								
Rest	100	(15)	0.78	(0.56 – 0.88)	0.81	(0.63 – 0.90)	-4	-25 – 17
PFMC	87	(18)	0.87	(0.75 – 0.93)	0.77	(0.54 – 0.88)	-6	-34 – 22
Overall*	93	(18)	0.86	(0.76 – 0.92)	0.82	(0.69 – 0.89)	-5	-29 - 20

n=number of datasets analyzed; PFMC=pelvic floor muscle contraction; ICC=intraclass correlation coefficient; 95%-CI=95%-confidence interval; MD=mean difference; LOA=limits of agreement; Overall=at rest and on PFMC together. *n=80

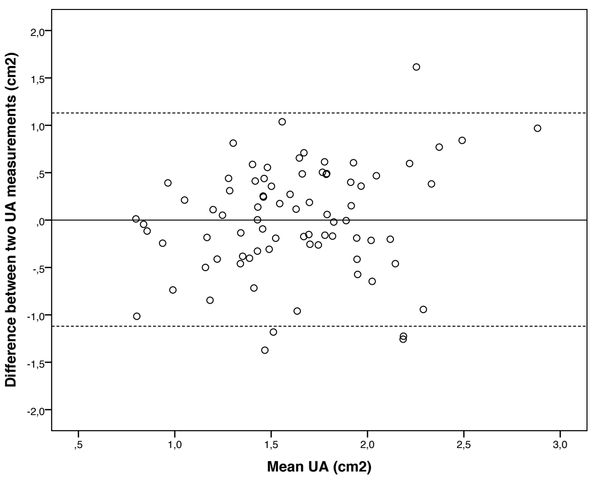


Figure 2. Bland-Altman plot of inter-observer reliability of the urethral area (UA). The dotted lines indicate limits of agreement. UA=urethral area

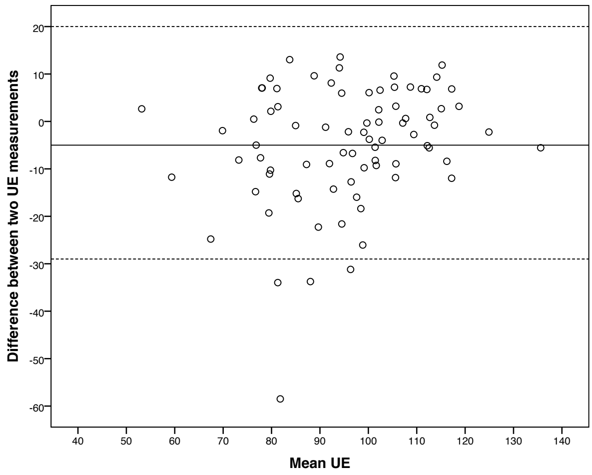


Figure 3. Bland-Altman plot of inter-observer reliability of the mean urethral echogenicity (UE). The dotted lines indicate limits of agreement. UE=urethral echogenicity

Patients

Of the 280 women recruited initially, a total of 26 patients were excluded. Reasons for exclusion were: incorrect inclusion (n = 2) (one with a neurological disorder and one with a twin pregnancy), immature labour at 19.9 weeks' of gestation (n = 1), loss to follow up after 12 weeks' of pregnancy (n = 17), and the symphysis was outside the view of the ultrasound image (n = 6). This left a dataset of 254 women. The number of women presented in the figures reflects those who had complete and adequate ultrasound recordings for this particular item.

Changes in mid-urethral area during pregnancy and after delivery

In Figure 4a the changes of the mean mid-urethral area of the women at 12 and 36 weeks of gestation and 6 months after delivery are shown graphically.

Mean mid-urethral area at rest was significantly smaller at 36 weeks of gestation when compared to 12 weeks of gestation ($p<0.05$), but not on contraction ($p=0.21$). At 6 months after delivery mid-urethral area was statistically significant smaller when compared to 12 and 36 weeks of gestation, both at rest ($p<0.05$) and on contraction ($p<0.05$). No statistical significant differences in the mean mid-urethral area after delivery were found between a vaginal delivery and caesarean section (area rest $p=0.47$; area contraction $p=0.06$).

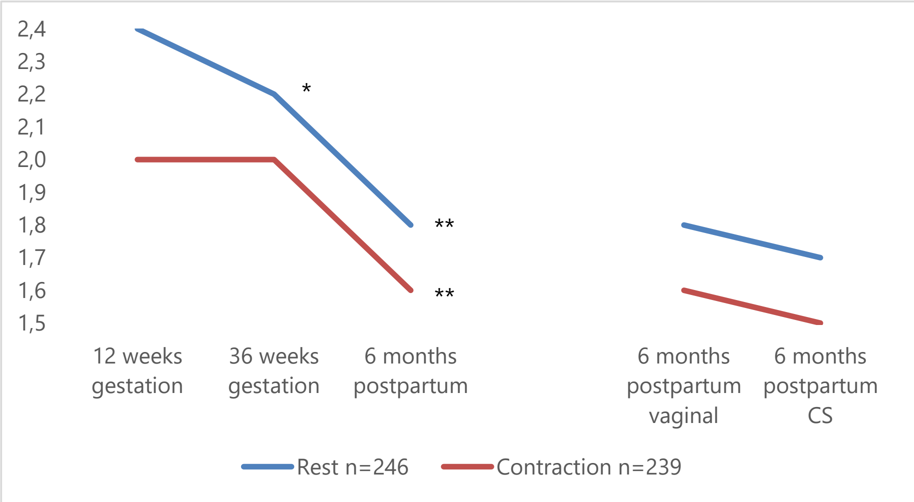


Figure 4a. Changes in mid-urethral area (cm²). *p≤0.05 compared to 12 weeks gestation; **p≤0.05 compared to 12 and 36 weeks gestation.

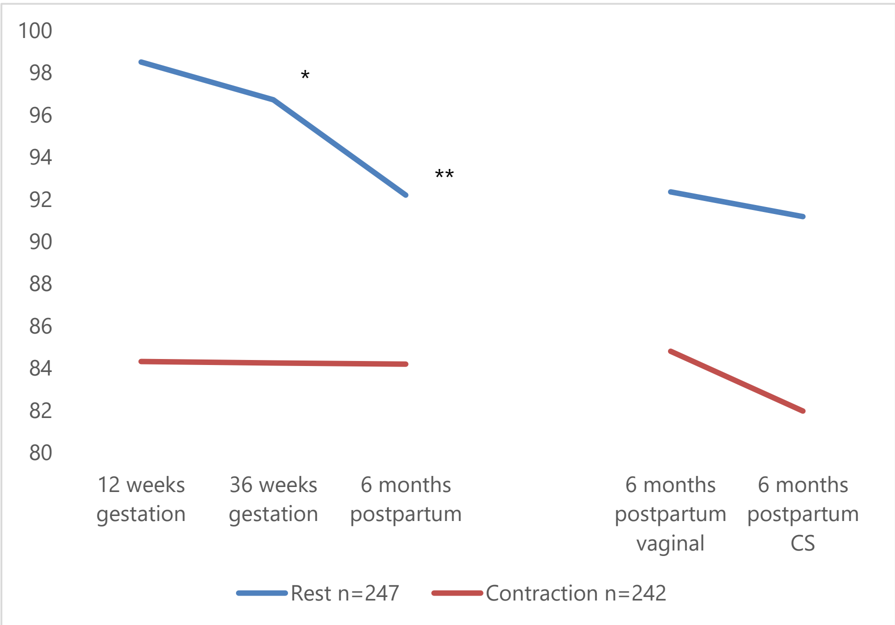


Figure 4b. Changes in mid-urethral mean echogenicity. *p≤0.05 compared to 12 weeks gestation; **p≤0.05 compared to 12 and 36 weeks gestation.

Changes in mid-urethral mean echogenicity during pregnancy and after delivery
In Figure 4b the changes of the mean mid-urethral echogenicity of the women at 12 and 36 weeks of gestation and 6 months after delivery are shown graphically.

Mean mid-urethral echogenicity did change significantly during pregnancy when the pelvic floor was at rest, but no difference was found when the pelvic floor was in contraction. A lower echogenicity was seen at 6 months after delivery when compared to 12 and 36 weeks of gestation, at rest ($p=0.00$ and $p=0.003$ respectively). No significant differences in mean echogenicity after delivery were found between a vaginal delivery and caesarean section (echogenicity rest $p=0.62$; echogenicity contraction $p=0.22$).

Discussion

This study shows that 3D/4D transperineal ultrasound can be reliably used to assess the mid-urethral area and mean echogenicity, as a representation of the urethral sphincter. The protocol shows almost perfect interobserver reliability for mid-urethral echogenicity, and moderate interobserver reliability for mid-urethral area. After delivery, area and mean echogenicity of the mid-urethra decreases significant as compared to 12 and 36 weeks pregnancy, except for the echogenicity during contraction. No significant differences in area and echogenicity are found between a vaginal delivery and caesarean section.

A strength of this research is the longitudinal follow up of the included women at 12 and 36 weeks gestational age and 6 months after delivery. And that the sample for the reliability study is picked at random and intraobserver reliability is performed in two separate randomized orders to prevent recall bias. A limitation of our study is the fact that we examine the urethral area instead of volume as done before[13,16,17,20] and that nulliparous women without muscular disease are included, resulting in high-quality ultrasound images.

Digesu and coworkers calculated urethral sphincter volumes based on the sum of multiple axial cross-sectional areas.[24] They demonstrated a good interobserver reliability of >0.6, which is comparable to our 0.59 in rest. It still has to be determined if the time consuming urethral volume measurements are clinically more relevant than single mid-urethral area measurements.[24]

Although we do not identify the urethral sphincter, it seems that assessing the area and echogenicity in the middle of the urethra will include the urethral sphincter, since various cadaver and imaging studies demonstrated that the urethral sphincter in females is located in the middle third of the urethra.[13,17,20,25-28] The presence of the urethral sphincter at this level is further supported by Robinson *et al.*, who observed maximal urethral pressure in the middle section of the urethra, using urethral pressure profilometry.[12]

We set out to study the area and echogenicity of the urethral striated muscle in rest and on contraction as possible markers of function. During pregnancy, the area in rest decreases significantly from 12 to 36 weeks gestational age and again after delivery, just like during contraction of the pelvic floor. This is in line with the observation that the echogenicity during pregnancy and after delivery decreases when the pelvic floor is in rest. Pregnancy, with its increased levels of progesterone, causes an increase in intracellular and intramuscular fat storage.[29] We hypothesize that this accumulation of fat also occurs in the urethral musculature, increasing its area and echogenicity during early pregnancy. Although the area in rest decreases in size between 12 and 36 weeks gestational age, this is not observed during contraction of the pelvic floor. This might be due to a weaker contraction at 36 weeks gestational age because of the extended gravidity and growing womb. In all comparison, during contraction of the pelvic floor, the urethral area decrease is most likely due to compression of the urethra to the pelvic fascia or it represents contraction of the external urethral sphincter itself. This is in line with the recently published study of Aljuraifani *et al.* who showed that the stiffness in the region of the urethral sphincter increased in all participants during contraction of the pelvic floor.[30] The fact that the echogenicity did not differ during contraction is mostly due to the little pressure needed to compress the ECM and represents the muscle fibers in the urethral sphincter. The decrease in echogenicity after delivery, when the pelvic floor is in rest, is consistent with the findings of Grob *et al.* in the puborectalis muscle.[31] If the ratio between muscle cells and ECM (which contains fat) changes towards less ECM, the echogenicity will decrease, in accordance with the decrease of the fat storage accumulation in pregnancy. Rat studies were used before to investigate the urethra during pregnancy and after delivery. The urethra of rats who are non-pregnant, rats who are pregnant and rats after a caesarean section showed a higher average concentration of muscle fibers compared to female rats with a normal vaginal delivery and rats who experienced a simulation of a delivery.[15] We assume that the lower average of muscle fibers in rats with birth trauma could represent a higher echogenicity (more EMC) which is opposite to the decrease in echogenicity in the resting urethra after delivery in the current study. However, we examined the women 6 months after delivery, the rats were examined directly after the trauma.

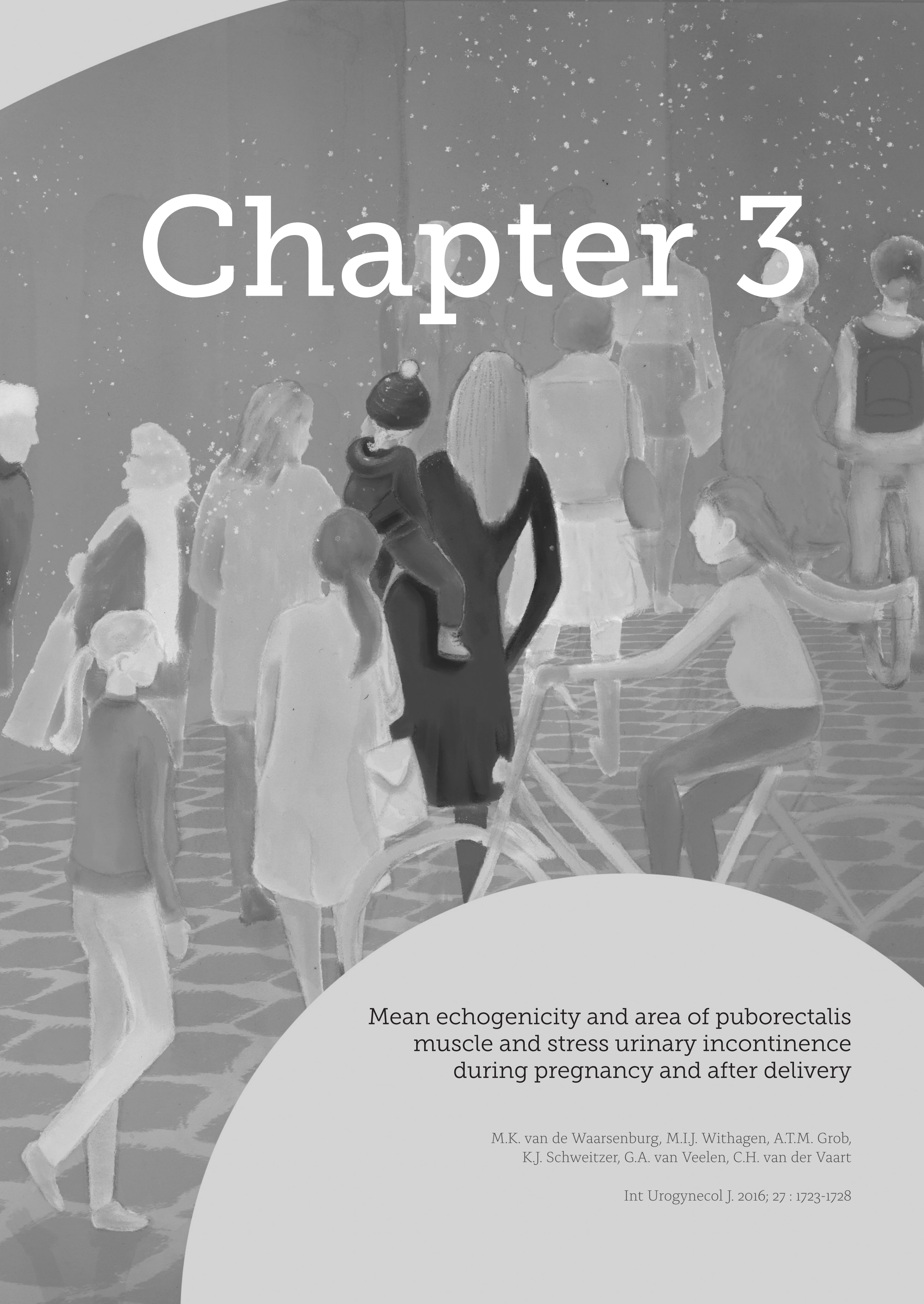
For a better understanding of the anatomy and function of the mid-urethra, especially in the continence mechanism, we are now able to measure the mid-urethral area (as surrogate marker for the volume) and mean echogenicity reliably and with a semi-automated method.

In conclusion, this study presents a reliable method to assess transverse mid-urethral area and mean echogenicity using transperineal ultrasound. With this protocol we were able to show that the area and echogenicity, except for the contraction, of the mid-urethra significantly decreased after pregnancy. The clinical merits of this finding, eg association with urinary incontinence, needs to be determined.

References

1. MacLennan AH, Taylor AW, Wilson DH, Wilson D. The prevalence of pelvic floor disorders and their relationship to gender, age, parity and mode of delivery. *BJOG* 2000; 107 : 1460-1470.
2. Rortveit G, Hannestad YS, Daltveit AK, Hunskaar S. Age- and type-dependent effects of parity on urinary incontinence: the Norwegian EPINCONT study. *Obstet Gynecol* 2001; 98 : 1004-1010.
3. Ashton-Miller JA, DeLancey JO. Functional anatomy of the female pelvic floor. *Ann N Y Acad Sci* 2007; 1101 : 266-296.
4. Dietz HP. Ultrasound imaging of the pelvic floor. Part II: three-dimensional or volume imaging. *Ultrasound Obstet Gynecol* 2004; 23 : 615-625.
5. Cosimato C, Cipullo LM, Troisi J, Di Spiezio Sardo A, Tommaselli GA, Oro RR, Zullo F, Altieri V, Guida M. Ultrasonographic evaluation of urethrovesical junction mobility: correlation with type of delivery and stress urinary incontinence. *Int Urogynecol J* 2015; 26 : 1495-1502.
6. Van Veelen GA, Schweitzer KJ, van der Vaart CH. Ultrasound imaging of the pelvic floor: changes in anatomy during and after first pregnancy. *Ultrasound Obstet Gynecol* 2014; 44 : 476-480.
7. Falkert A, Willmann A, Endress E, Meint P, Seelbach-Gobel B. Three-dimensional ultrasound of pelvic floor: is there a correlation with delivery mode and persisting pelvic floor disorders 18-24 months after first delivery? *Ultrasound Obstet Gynecol* 2013; 41 : 204-209.
8. Wijma J, Weis Potters AE, de Wolf BT, Tinga DJ, Aarnoudse JG. Anatomical and functional changes in the lower urinary tract during pregnancy. *BJOG* 2001; 108 : 726-732.
9. Jundt K, Scheer I, Schiessl B, Karl K, Friese K, Peschers UM. Incontinence, bladder neck mobility, and sphincter ruptures in primiparous women. *Eur J Med Res* 2010; 15 : 246-252.
10. Shek KL, Kruger J, Dietz HP. The effect of pregnancy on hiatal dimensions and urethral mobility: an observational study. *Int Urogynecol J* 2012; 23 : 1561-1567.
11. DeLancey JO, Miller JM, Kearney R, Howard D, Reddy P, Umek W, Guire KE, Margulies RU, Ashton-Miller JA. Vaginal birth and de novo stress incontinence: relative contributions of urethral dysfunction and mobility. *Obstet Gynecol* 2007; 110 : 354-362.
12. Robinson D, Toozs-Hobson P, Cardozo L, Digesu A. Correlating structure and function: three-dimensional ultrasound of the urethral sphincter. *Ultrasound Obstet Gynecol* 2004; 23 : 272-276.
13. Klauser A, Frauscher F, Strasser H, Helweg G, Kolle D, Strohmeyer D, Stenzl A, zur Nedden D. Age-related rhabdosphincter function in female urinary stress incontinence: assessment of intraurethral sonography. *J Ultrasound Med* 2004; 23 : 631-7; quiz 638-9.
14. Prantil-Baun R, de Groat WC, Miyazato M, Chancellor MB, Yoshimura N, Vorp DA. Ex vivo biomechanical, functional, and immunohistochemical alterations of adrenergic responses in the female urethra in a rat model of birth trauma. *Am J Physiol Renal Physiol* 2010; 299 : F316-24.
15. Rocha MA, Sartori MG, De Jesus Simoes M, Herrmann V, Baracat EC, Rodrigues de Lima G, Girao MJ. The impact of pregnancy and childbirth in the urethra of female rats. *Int Urogynecol J Pelvic Floor Dysfunct* 2007; 18 : 645-651.
16. Athanasiou S, Khullar V, Boos K, Salvatore S, Cardozo L. Imaging the urethral sphincter with three-dimensional ultrasound. *Obstet Gynecol* 1999; 94 : 295-301.
17. Kondo Y, Homma Y, Takahashi S, Kitamura T, Kawabe K. Transvaginal ultrasound of urethral sphincter at the mid urethra in continent and incontinent women. *J Urol* 2001; 165 : 149-152.
18. Pillen S, van Alfen N. Skeletal muscle ultrasound. *Neurol Res* 2011; 33 : 1016-1024.

19. Grob AT, Veen AA, Schweitzer KJ, Withagen MI, van Veelen GA, van der Vaart CH. Measuring echogenicity and area of the puborectalis muscle: method and reliability. *Ultrasound Obstet Gynecol* 2014; 44 : 481-485.
20. Mitterberger M, Pinggera GM, Mueller T, Frauscher F, Pallwein L, Gradl J, Peschel R, Bartsch G, Strasser H. Dynamic transurethral sonography and 3-dimensional reconstruction of the rhabdosphincter and urethra: initial experience in continent and incontinent women. *J Ultrasound Med* 2006; 25 : 315-320.
21. Scholten RR, Pillen S, Verrips A, Zwarts MJ. Quantitative ultrasonography of skeletal muscles in children: normal values. *Muscle Nerve* 2003; 27 : 693-698.
22. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; 33 : 159-174.
23. Myles PS, Cui J. Using the Bland-Altman method to measure agreement with repeated measures. *Br J Anaesth* 2007; 99 : 309-311.
24. Digesu GA, Calandrini N, Derpapas A, Gallo P, Ahmed S, Khullar V. Intraobserver and interobserver reliability of the three-dimensional ultrasound imaging of female urethral sphincter using a translabial technique. *Int Urogynecol J* 2012; 23 : 1063-1068.
25. Elbadawi A. Functional anatomy of the organs of micturition. *Urol Clin North Am* 1996; 23 : 177-210.
26. Preyer O, Brugger PC, Laml T, Hanzal E, Prayer D, Umek W. High resolution magnetic resonance imaging of urethral anatomy in continent nulliparous pregnant women. *Eur J Radiol* 2011; 80 : 736-739.
27. Toozs-Hobson P, Balmforth J, Cardozo L, Khullar V, Athanasiou S. The effect of mode of delivery on pelvic floor functional anatomy. *Int Urogynecol J Pelvic Floor Dysfunct* 2008; 19 : 407-416.
28. Umek WH, Kearney R, Morgan DM, Ashton-Miller JA, DeLancey JO. The axial location of structural regions in the urethra: a magnetic resonance study in nulliparous women. *Obstet Gynecol* 2003; 102 : 1039-1045.
29. Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *N Engl J Med* 2014; 371 : 2237-2238.
30. Aljuraifani R, Stafford RE, Hug F, Hodges PW. Female striated urogenital sphincter contraction measured by shear wave elastography during pelvic floor muscle activation: Proof of concept and validation. *Neurourol Urodyn* 2017; .
31. Grob AT, Withagen MI, van de Waarsenburg MK, Schweitzer KJ, van der Vaart CH. Changes in the mean echogenicity and area of the puborectalis muscle during pregnancy and postpartum. *Int Urogynecol J* 2016; 27 : 895-901.



Chapter 3

Mean echogenicity and area of puborectalis muscle and stress urinary incontinence during pregnancy and after delivery

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Int Urogynecol J. 2016; 27 : 1723-1728

Abstract

Introduction: Pregnancy and childbirth are risk factors for the development of stress urinary incontinence (SUI). Urinary continence depends on normal urethral support which is provided by normal levator ani muscle function. Our objective was to compare mean echogenicity and area of the puborectalis muscle between women with and without SUI during and after their first pregnancy.

Methods: We examined 280 nulliparous women at a gestational age of 12 weeks, 36 weeks and 6 months after delivery. They filled out the validated Urogenital Distress Inventory and underwent perineal ultrasounds. SUI was considered present if the woman answered positively to the question "do you experience urine leakage related to physical activity, coughing, or sneezing?". Mean Echogenicity of the puborectalis muscle (MEP) and puborectalis muscle area (PMA) were calculated. The MEP and PMA during pregnancy and after delivery between women with and without SUI were compared using independent Student's t-test.

Results: After delivery the MEP was higher in women with SUI if the pelvic floor was at rest or in contraction, with effect sizes of 0.30 and 0.31 respectively. No difference in area of the puborectalis muscle between women with and without SUI was found.

Conclusion: Women with SUI after delivery had a statistical significant higher mean echogenicity of the puborectalis muscle as compared to non-SUI women when the pelvic floor was at rest and in contraction, the effect sizes were small. This higher MEP is indicative of a relatively higher intramuscular extracellular matrix component and could represent diminished contractile function.

Introduction

Pregnancy and childbirth are risk factors for the development of stress urinary incontinence (SUI).[1,2] Urinary continence depends on normal urethral support which is provided by normal levator ani muscle function, more specific the puborectalis muscle part, and an intact endopelvic fascia. Damage to this urethral support during vaginal delivery can result in loss of function and an increased mobility of the bladder neck[3-5] which is the main contributing factor to SUI.[6]

During pregnancy SUI has been associated with the width of the hiatal area, and after delivery with the positioning of the bladder neck.[5] The observation that during pregnancy a large hiatal area is associated with SUI raises the question if this is related to structural abnormalities of the puborectalis muscle, which forms the boundaries of the genital hiatus. Normal functioning of a muscle is, among others, dependent on its volume and structural integrity. Regarding true volume measurements of the puborectalis muscle we would need adequate 3-dimensional delineation which is not readily available. In 2-D planes the area of the puborectalis muscle can be assessed and, within its limitations, be indicative of the volume. With respect to the structure of muscles we know that atrophic muscles and muscles with increasing extracellular matrix (ECM) content (collagen) have poor contractility force.[7] This structural composition of a muscle can be indirectly assessed with ultrasound, especially by measuring echogenicity.[8] Echogenicity of the muscle represents the ratio between muscle cells and ECM, and was recently shown to change during pregnancy and after delivery (unpublished data).

In this study we set out to assess the association between the puborectalis muscle area (PMA) and SUI symptoms and the association between the mean echogenicity of the puborectalis muscle (MEP) and SUI symptoms during and after first pregnancy.

Methods

This study is a secondary analysis of a prospective observational study on the association between pelvic floor symptoms and changes in pelvic floor anatomy during and after first pregnancy.[5] Two hundred eighty nulliparous women with a singleton pregnancy and good knowledge of the Dutch language were included in the original study. Exclusion criteria were a medical history of urinary or fecal incontinence, prolapse or anti-incontinence surgery, connective tissue diseases, neurological disorders and an inability to perform a maximum Valsalva maneuver because of cardiac or pulmonary disease. The Institutional Human Research Ethics Committee approved the study (reference 08/299) and all women gave informed consent.

The participants were invited for 3D/4D transperineal ultrasound examination at a gestational age of 12 weeks and 36 weeks and 6 months after delivery. The examinations were performed by two observers, one of the observers had 6 years' experience with 3D/4D transperineal ultrasound and the other observer was trained by the experienced observer. We have previously published data on their intra- and interobserver reliability[9]. A GE Voluson 730 Expert ultrasound system (GE Healthcare, Hoevelaken, the Netherlands) with a RAB 4-8 MHz curved array 3D/4D ultrasound transducer was used. It was crucial that the intensity values were kept constant as described by Scholten *et al.*[10] We used gain 15, power 100, Harmonics mid, contrast 8, grey map 4, persistence 8, and enhance 3. The women had an empty bladder. Volume imaging datasets were obtained at rest, on maximum pelvic floor muscle contraction and on maximum Valsalva maneuver.

After storage on a hard disk, offline analysis was performed using the 4D View 7.0 (GE Medical Systems Kretztechnik, Zipf, Austria) and Matlab® R2010a (MathWorks, Natick, MA) software. The plane of minimal hiatal dimensions in axial position was selected and exported as previously described by Dietz.[11] A semi-automated method to delineate the puborectalis muscle and measure PMA and MEP was used. This method was previously tested and proved to be reliable.[12]

Pelvic floor symptoms and physical complaints were scored at every ultrasound examination. SUI was present when a woman answered positively to the Urogenital Distress Inventory question "do you experience urine leakage related to physical activity, coughing, or sneezing".[13,14]

The association between the MEP and Body Mass Index (before pregnancy), the mode of delivery (vaginal vs caesarean section), the duration of the second stage of labour (<60 min and >=60 min), the use of oxytocin (yes/no) during delivery, the mean birth weight and the use of pain relief (drugs or epidural) were assessed for potential confounding effect.

Statistical analysis was performed using SPSS version 20.0 for Windows. The MEP and PMA during pregnancy and after delivery between women with and without SUI were compared using independent Student's t-test. Statistical significance was based on two-sided tests, with $p<0.05$ considered significant. To determine the magnitude of the effect we calculated the effect size of the statistically significant findings by Cohen's d.

Results

Of the 280 women, 26 cases were excluded, leaving 254 women to be studied. Excluded were women with an incorrect inclusion because of a twin pregnancy (n=1) and a neurological disorder (n=1). Other reasons for exclusion were immature labor at 19.9 weeks' gestation (n=1), loss to follow-up and/or at least one out of three ultrasound volume datasets (rest, contraction or Valsalva) missing (n=17), and the symphyses was located outside the view of the ultrasound images (n=6).

The flowchart (Figure 1) shows the distribution of women with complete datasets that could be analyzed for each timeframe. Patient characteristic are described in Table 1.

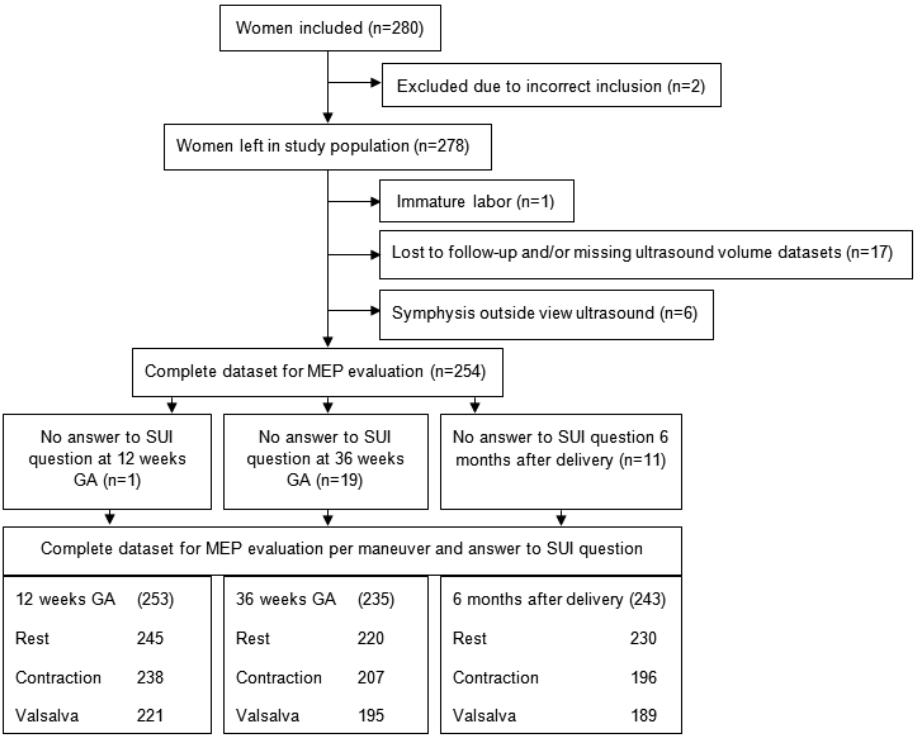


Figure 1. Flow chart. n=number; SUI=Stress Urinary Incontinence; GA=gestational age.

Table 1. Patient characteristics

Variables	n=254
Age at first US, years; mean (SD)	31.1 (4.1)
BMI at first US, kg/m^2; median (range)	22.4 (17.9-40.4)
Gestational age at first US, wk; median (range)	13.0 (8.4-21.0)
Time period after delivery at third visit, wk; median (range)	27.0 (23.6-57.4)
Gestational age at delivery, wk; median (range)	40.2 (33.0-42.6)
Delivery mode; n (%)	249
Spontaneous vaginal	157 (63.1)
Operative vaginal	45 (18.1)
Elective CS	11 (4.4)
Emergency CS	36 (14.4)
Birth weight, g; median (range)	3365 (1590-4750)

n=number; SD=standard deviation; US=ultrasound; kg=kilogram; m=meter, wk=weeks; CS=caesarean section; g=gram

The relation between MEP and SUI is shown in Table 2. During pregnancy no statistical significant differences in the mean echogenicity of the women with SUI and without SUI at the different maneuvers were found. Six months after delivery there was a statistical significant higher MEP in women with SUI compared to women without SUI when the pelvic floor was at rest (p = 0.03) and when the pelvic floor was in contraction (p = 0.04), with effect sizes of 0.30 and 0.31 respectively.

The relation between PMA and SUI is shown in Table 3. No significant differences were found in the PMA during pregnancy and after delivery in women with and without SUI.

Table 2. MEP in relation to SUI during pregnancy and after delivery

	Women with SUI		Women without SUI		
Gestational age and maneuver (n)	n (%)	MEP mean ± SD	n (%)	MEP mean ± SD	p-value
12 weeks (253)	44 (17.4)		209 (82.6)		
Rest (245)	44	141.0 ± 20.6	201	141.0 ± 20.1	0.99
Contraction (238)	43	136.3 ± 22.3	195	132.2 ± 20.6	0.25
Valsalva (221)	38	134.7 ± 21.9	183	134.7 ± 21.2	0.99
36 weeks (235)	112 (47.7)		123 (52.3)		
Rest (220)	104	147.0 ± 19.8	116	149.2 ± 19.8	0.42
Contraction (207)	97	138.1 ± 19.9	110	139.0 ± 21.9	0.76
Valsalva (195)	89	133.6 ± 24.6	106	135.1 ± 21.7	0.65
6 months after delivery (244)	90 (36.9)		154 (63.1)		
Rest (230)	***87***	***132.1 ± 20.9***	***143***	***126.2 ± 19.8***	***0.03***
Contraction (196)	***72***	***125.6 ± 23.7***	***124***	***118.6 ± 22.6***	***0.04***
Valsalva (189)	71	118.7 ± 22.0	118	115.0 ± 21.3	0.25

SUI=Stress Urinary Incontinence; MEP=Mean Echogenicity of the Puborectalis muscle; SD=standard deviation.

Table 3. PMA in relation to SUI during pregnancy and after delivery

	Women with SUI		Women without SUI		
Gestational age and maneuver (n)	n (%)	PMA (cm^2) mean ± SD	n (%)	PMA (cm^2) mean ± SD	p-value
12 weeks (253)	44 (17.4)		209 (82.6)		
Rest (245)	44	5.9 ± 1.4	201	5.6 ± 1.3	0.12
Contraction (235)	42	5.5 ± 1.2	193	5.0 ± 1.2	0.11
Valsalva (222)	38	6.0 ± 1.5	184	5.8 ± 1.3	0.32
36 weeks (235)	112 (47.7)		123 (52.3)		
Rest (219)	104	5.9 ± 1.4	115	5.8 ± 1.2	0.82
Contraction (207)	97	5.4 ± 1.2	110	5.2 ± 1.3	0.27
Valsalva (194)	89	6.6 ± 1.5	105	6.2 ± 1.3	0.10
6 months after delivery (244)	90 (36.9)		154 (63.1)		
Rest (230)	87	5.6 ± 1.2	143	5.4 ± 1.5	0. 43
Contraction (192)	69	5.2 ± 1.2	123	4.8 ± 1.4	0.10
Valsalva (191)	72	5.9 ± 1.4	119	5.9 ± 1.5	0.86

SUI=Stress Urinary Incontinence; PMA=Puborectalis Muscle Area; SD=standard deviation
None of the potential confounding factors were significantly associated with the MEP.

Discussion

We set out to assess the association between MEP and PMA and SUI during and after first pregnancy. We found that the MEP in women with SUI after delivery was statistically significant higher as compared to stress urinary continent women. However, effect sizes were low, indicating that the clinical relevance is questionable and that MEP cannot be used to differentiate women with SUI from those without.

A possible limitation of our study is the absence of pre-pregnancy clinical and ultrasound data. We were only able to look at associations between SUI and MEP and PMA at different time points during and after pregnancy. Changes in MEP and PMA that occurred between pre-pregnant and early pregnant status may have provided extra information on the association between these parameters and SUI. We know from epidemiological studies that childbirth is the major risk factor for developing stress urinary incontinence symptoms. Therefore, we feel it is not an obvious limitation to look at the association between stress urinary incontinence symptoms and ultrasound findings postpartum without having knowledge of pre-pregnancy data. Another limitation is the fact that we had to use the PMA as a surrogate marker for puborectalis muscle volume.

The presence of levator avulsions could be a cause of a smaller PMA since the avulsion area, which is darker, would not have been incorporated into our semiautomatic muscle outline method. However, we previously demonstrated that the reliability of detecting levator avulsions in this particular population of postpartum women, when assessed in a multicenter multiobserver setting, is poor.[15] This showed us that assessing levator avulsions in the population under study cannot be reliably done and therefore we considered it inappropriate to use it as a potential confounding factor in our present paper.

We used a symptom based assessment of SUI according to the ICS standardization, in line with a previous study.[16,17] We did not consider it appropriate to perform a standardized stress test in pregnant women to confirm incontinence as a sign or to perform multichannel urodynamics to confirm incontinence as a condition. Our results have to be viewed from this symptom based stress SUI perspective.

The strengths of this study are the prospective design and the use of identical ultrasound settings during the examinations, which made echogenicity analyses possible.

The higher MEP, i.e. brighter muscle on ultrasound image, is indicative of a change in muscle tissue composition. The ratio between muscle cells and ECM expresses itself in the echogenicity (grey-scale) values on ultrasound.[8] Muscle cells appear dark on ultrasound whereas the extracellular matrix (ECM), containing mainly collagen and fat, appears bright. An increase in echogenicity has been associated with disease progression in children with neuromuscular disease,[8] and was shown to be associated with a decrease in muscle strength.[18] Echogenicity of a muscle increases with ageing and after major or recurrent minor injuries.[19-21] Muscle injuries can lead to scar formation and loss of contractility function.[20,21] Our finding that the MEP after delivery in women with SUI is higher as compared to non-SUI women indicates that the muscle composition has changed in favor of the ECM. This may affect the contractile force of the puborectalis muscle and diminish urethral support, which may present itself as hypermobility of the urethra and consequently SUI. We did not observe differences in MEP between SUI and non-SUI women during pregnancy. The obvious reason is that delivery injury has not yet occurred and no scar tissue, affecting echogenicity, has been formed.

The PMA was not related to SUI, whereas the hiatal area in a previous analysis of our data was.[5] The hypothesis was that a larger hiatal area is associated with a smaller sized puborectalis muscle. In that respect we would expect that SUI also was associated with a smaller sized puborectalis muscle. Our observation that there is no association between SUI and PMA is limited by the fact that we did not measure the true volume of the puborectalis muscle, which would have needed accurate 3-D volume information. Since muscle volume is associated with muscle strength,[22] the absence of an association between the PMA and SUI has to be interpreted with caution.

We previously demonstrated that SUI was associated with a larger hiatal area during pregnancy, and with a more dorsal and caudal positioning of the bladder neck after childbirth.[5] We suggested that the pathophysiology of SUI was different during pregnancy compared to pathophysiology after delivery. This hypothesis is challenged by other groups who demonstrated different associations from ours between SUI and the hiatal area or bladder neck positioning both during and after pregnancy.[23,24] However, our current findings on the MEP support the view that the mechanism behind SUI is different during pregnancy from that after delivery.

Although statistical significant, the difference between the MEP in women with SUI and those without was small. This might be related to the moment of scanning which was 6 months after delivery. We do not know how many women were breastfeeding or had returned to their normal menstrual cycle by that time point.[25,26] Estrogens play an important role in the wound healing process and low estrogen status, as in ageing, is associated with poorer healing.[27] Therefore, depending on their estrogen status, women may have been in different stages of recovery from their delivery trauma during the time of scanning. In fully developed scar tissue the echogenicity of the scar tissue does not change between rest and contraction.[20] However, our data show that there is a difference in MEP between rest and contraction in SUI women. This may indicate that the trauma to the puborectalis muscle was mild with little scar tissue formation,[28] or that the complete scare tissue formation in major trauma has not yet fully occurred. We could not demonstrate a significant association between the MEP at Valsalva after delivery between SUI and non-SUI women. This may be caused by the fact that the Valsalva maneuver, or pushing, is passive stretching of the muscle. In contrast what happens with muscle echogenicity during contraction, little information is available on Valsalva. One of the reasons may be that performing a true Valsalva maneuver is much more difficult than performing a contraction. There will be large individual differences which will affect the data obtained on MEP and PMA. In addition, Dietz and coworkers showed that performing a maximal Valsalva maneuver requires long forceful bearing down.[29] Especially during pregnancy and after delivery women may be reluctant to do so.

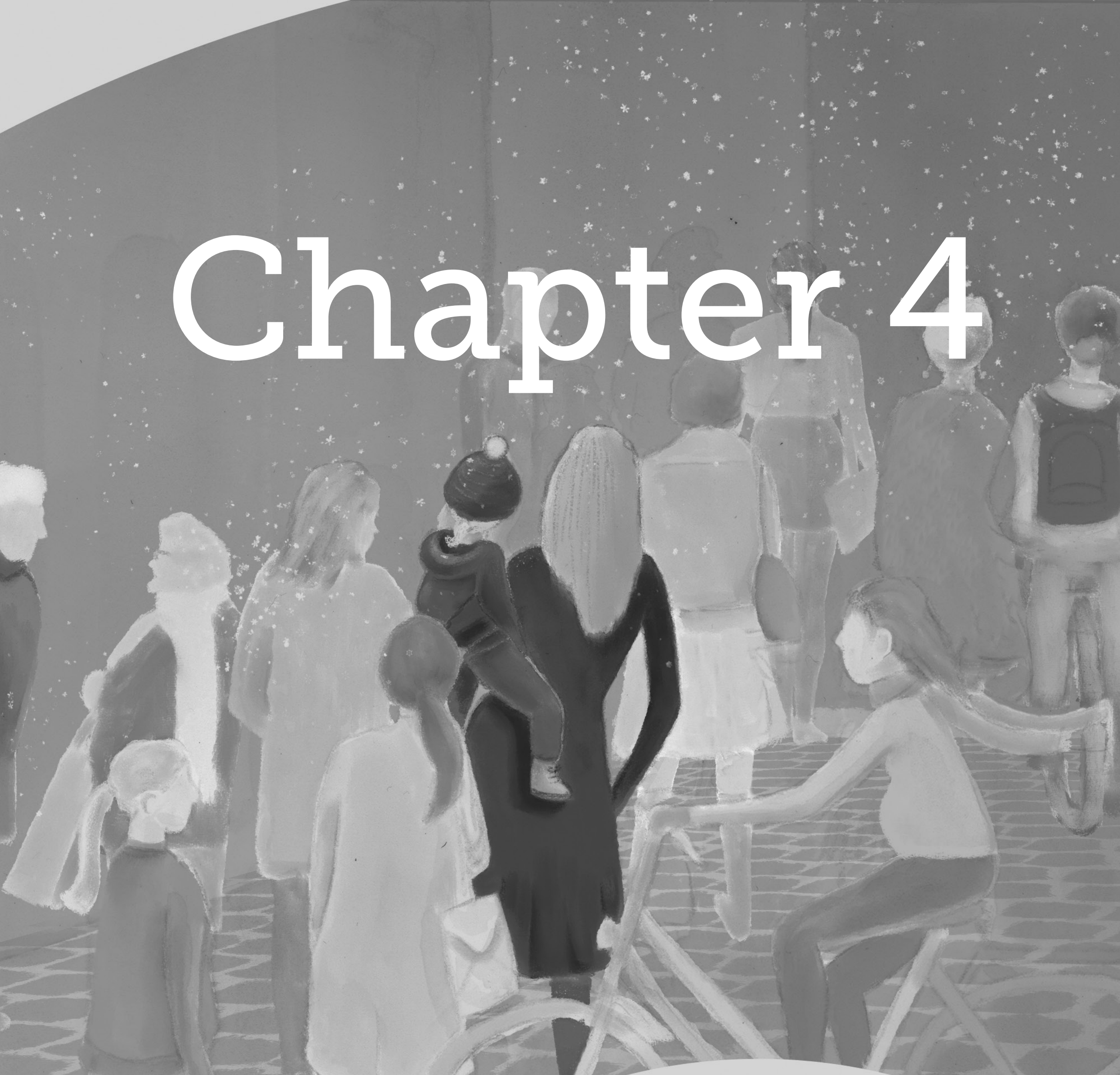
In conclusion, women with SUI after delivery where shown to have a statistical significant higher MEP as compared to non-SUI women when the pelvic floor was at rest and in contraction, although the effect sizes were small.

This higher MEP is indicative of a relatively higher intramuscular ECM component and could represent diminished contractile function.

References

1. MacLennan AH, Taylor AW, Wilson DH, Wilson D. The prevalence of pelvic floor disorders and their relationship to gender, age, parity and mode of delivery. *BJOG* 2000; 107 : 1460-1470.
2. Rortveit G, Hannestad YS, Daltveit AK, Hunskaar S. Age- and type-dependent effects of parity on urinary incontinence: the Norwegian EPINCONT study. *Obstet Gynecol* 2001; 98 : 1004-1010.
3. Dietz HP, Steensma AB. The prevalence of major abnormalities of the levator ani in urogynaecological patients. *BJOG* 2006; 113 : 225-230.
4. Delancey JO. Fascial and muscular abnormalities in women with urethral hypermobility and anterior vaginal wall prolapse. *Am J Obstet Gynecol* 2002; 187 : 93-98.
5. Van Veelen A, Schweitzer K, van der Vaart H. Ultrasound assessment of urethral support in women with stress urinary incontinence during and after first pregnancy. *Obstet Gynecol* 2014; 124 : 249-256.
6. Rortveit G, Daltveit AK, Hannestad YS, Hunskaar S, Norwegian EPINCONT Study. Urinary incontinence after vaginal delivery or cesarean section. *N Engl J Med* 2003; 348 : 900-907.
7. Oatis CA. *Kinesiology: The Mechanics and Pathomechanics of Human Movement*. Lippincott Williams \& Wilkins, 2004.
8. Pillen S, van Alfen N. Skeletal muscle ultrasound. *Neurol Res* 2011; 33 : 1016-1024.
9. Van Veelen GA, Schweitzer KJ, van der Vaart CH. Reliability of pelvic floor measurements on three- and four-dimensional ultrasound during and after first pregnancy: implications for training. *Ultrasound Obstet Gynecol* 2013; 42 : 590-595.
10. Scholten RR, Pillen S, Verrips A, Zwarts MJ. Quantitative ultrasonography of skeletal muscles in children: normal values. *Muscle Nerve* 2003; 27 : 693-698.
11. Dietz HP, Shek C, Clarke B. Biometry of the pubovisceral muscle and levator hiatus by three-dimensional pelvic floor ultrasound. *Ultrasound Obstet Gynecol* 2005; 25 : 580-585.
12. Grob AT, Veen AA, Schweitzer KJ, Withagen MI, van Veelen GA, van der Vaart CH. Measuring echogenicity and area of the puborectalis muscle: method and reliability. *Ultrasound Obstet Gynecol* 2014; 44 : 481-485.
13. Shumaker SA, Wyman JF, Uebersax JS, McClish D, Fantl JA. Health-related quality of life measures for women with urinary incontinence: the Incontinence Impact Questionnaire and the Urogenital Distress Inventory. Continence Program in Women (CPW) Research Group. *Qual Life Res* 1994; 3 : 291-306.
14. Van der Vaart CH, de Leeuw JR, Roovers JP, Heintz AP. Measuring health-related quality of life in women with urogenital dysfunction: the urogenital distress inventory and incontinence impact questionnaire revisited. *Neurourol Urodyn* 2003; 22 : 97-104.
15. Van Veelen GA, Schweitzer KJ, van Delft K, Kluivers KB, Weemhoff M, van der Vaart CH. Diagnosing levator avulsions after first delivery by tomographic ultrasound: reliability between observers from different centers. *Int Urogynecol J* 2014; 25 : 1501-1506.
16. Haylen BT, de Ridder D, Freeman RM, Swift SE, Berghmans B, Lee J, Monga A, Petri E, Rizk DE, Sand PK, Schaer GN. An International Urogynecological Association (IUGA)/International Continence Society (ICS) joint report on the terminology for female pelvic floor dysfunction. *Int Urogynecol J* 2010; 21 : 5-26.
17. Labrie J, Berghmans BL, Fischer K, Milani AL, van der Wijk I, Smalbraak DJ, Vollebregt A, Schellart RP, Graziosi GC, van der Ploeg JM, Brouns JF, Tiersma ES, Groenendijk AG, Scholten P, Mol BW, Blokhuis EE, Adriaanse AH, Schram A, Roovers JP, Lagro-Janssen AL, van der Vaart CH. Surgery versus physiotherapy for stress urinary incontinence. *N Engl J Med* 2013; 369 : 1124-1133.

18. Jansen M, van Alfen N, Nijhuis van der Sanden MW, van Dijk JP, Pillen S, de Groot IJ. Quantitative muscle ultrasound is a promising longitudinal follow-up tool in Duchenne muscular dystrophy. *Neuromuscul Disord* 2012; 22 : 306-317.
19. Watanabe Y, Yamada Y, Fukumoto Y, Ishihara T, Yokoyama K, Yoshida T, Miyake M, Yamagata E, Kimura M. Echo intensity obtained from ultrasonography images reflecting muscle strength in elderly men. *Clin Interv Aging* 2013; 8 : 993-998.
20. Lee JC, Healy J. Sonography of lower limb muscle injury. *AJR Am J Roentgenol* 2004; 182 : 341-351.
21. Vanhoenacker FM, Baert AL, Faletti C, Maas M, Gielen JL. *Imaging of Orthopedic Sports Injuries*. Springer Berlin Heidelberg, 2007.
22. Holzbaur KR, Delp SL, Gold GE, Murray WM. Moment-generating capacity of upper limb muscles in healthy adults. *J Biomech* 2007; 40 : 2442-2449.
23. Falkert A, Willmann A, Endress E, Meint P, Seelbach-Gobel B. Three-dimensional ultrasound of pelvic floor: is there a correlation with delivery mode and persisting pelvic floor disorders 18-24 months after first delivery? *Ultrasound Obstet Gynecol* 2013; 41 : 204-209.
24. Chan SS, Cheung RY, Yiu KW, Lee LL, Leung TY, Chung TK. Pelvic floor biometry during a first singleton pregnancy and the relationship with symptoms of pelvic floor disorders: a prospective observational study. *BJOG* 2014; 121 : 121-129.
25. Hausswirth C, Le Meur Y. Physiological and nutritional aspects of post-exercise recovery: specific recommendations for female athletes. *Sports Med* 2011; 41 : 861-882.
26. Tiidus PM, Deller M, Bombardier E, Gul M, Liu XL. Estrogen supplementation failed to attenuate biochemical indices of neutrophil infiltration or damage in rat skeletal muscles following ischemia. *Biol Res* 2005; 38 : 213-223.
27. Ashcroft GS, Ashworth JJ. Potential role of estrogens in wound healing. *Am J Clin Dermatol* 2003; 4 : 737-743.
28. Takebayashi S, Takasawa H, Banzai Y, Miki H, Sasaki R, Itoh Y, Matsubara S. Sonographic findings in muscle strain injury: clinical and MR imaging correlation. *J Ultrasound Med* 1995; 14 : 899-905.
29. Mulder FE, Shek KL, Dietz HP. What's a proper push? The Valsalva maneuver revisited. *Aust N Z J Obstet Gynaecol* 2012; 52 : 282-285.



Chapter 4

Echogenicity of the puborectalis muscle, the cervix and the vastus lateralis muscle in pregnancy in relation to mode of delivery

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J.H. Schagen van Leeuwen, C.H. van der Vaart

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Abstract

Objectives: The primary objective of this study was to confirm our previous observation that hiatal dimensions and mean echogenicity of the puborectalis muscle (MEP) were statistically significant different at 12 weeks gestation in women who delivered by a caesarean section (CS) due to failure to progress (FTP), compared to women who delivered vaginally. The secondary objective was to assess the association between the echogenicity of the cervix and the vastus lateralis muscle and mode of delivery.

Methods: In this multicenter study 306 nulliparous women with a singleton pregnancy received ultrasound assessments of the pelvic floor at rest, contraction and on Valsalva maneuver, of the cervix and of the vastus lateralis muscle at 12 weeks' gestation. Dimensions of the levator hiatus and mean echogenicity of the puborectalis muscle, cervix and vastus lateralis muscle were measured.

Results: Two hundred forty-seven women were eligible for analyses. We were unable to confirm our previous finding that the MEP and hiatal transverse diameter and area at 12 weeks gestation are significantly associated with the mode of delivery. In addition, we could not demonstrate a significant association between the echogenicity of the cervix and vastus lateralis muscle and mode of delivery. Furthermore, no significant differences between the levator hiatal dimensions and the modes of delivery are found. Overall, we noticed a mean 20 points lower MEP in all women in the new database as compared to the previous study, despite the use of the same ultrasound equipment.

Conclusion: In a second, independent multicenter dataset we were unable to confirm our previous finding that the hiatal dimensions and MEP on contraction were statistically significant associated with the mode of delivery. The overall lower MEP in all women was caused by to a higher depth in all ultrasound images of the current study.

Introduction

Most Caesarean sections (CS) are performed because of failure to progress (FTP) or for suspected fetal distress (FD) in term pregnancies with the fetus in cephalic presentation.[1,2] Several clinical ante- and intra-partum characteristics have been associated with the mode of delivery.[3-7] Using these characteristics, predictive models have been developed to council women for their expected mode of delivery.[8-14] However, pelvic floor muscle quality has never been part of such a predictive model.

The levator ani muscle plays an important role in delivery. In order to allow passage of the fetal head it has to stretch up to 250% of its original length.[15] It may well be that abnormalities in levator ani structure or function are associated with failure to deliver vaginally. The function of the levator ani can be assessed by measuring the hiatal dimensions of the levator ani muscle. The structure of the levator ani can be assessed using greyscale analyses of ultrasound images, e.g. its echogenicity.[16-18] In a previous study we have shown that women who delivered by CS due to FTP, had a significantly smaller transverse diameter and hiatal area of the levator ani hiatus during muscle contraction at 12 weeks' pregnancy, compared to women who delivered spontaneously.[19] In addition, the mean echogenicity of the puborectalis muscle (MEP) during contraction at 12 weeks gestation was statistically significant lower.[20] However, the number of women with a CS due to FTP was limited and the difference in MEP was only observed during contraction. The primary aim of this study was to confirm our previous observation that hiatal dimensions and MEP were already statistically significant different at 12 weeks gestation in women who finally delivered by a CS due to FTP.

The effect of pregnancy on muscle tissue composition may also differ between the pelvic floor and non-pelvic floor muscles. For instance, rats showed an increase in the extra cellular matrix (ECM) of the pelvic floor muscles during pregnancy, while the ECM of the tibialis anterior muscle did not differ.[21,22] The secondary aim of our study was therefore to assess in pregnancy the association between the echogenicity of the cervix and the vastus lateralis muscle and mode of delivery.

Methods

This study is a prospective multicenter cohort study in the Netherlands on the association between the mean echogenicity of the puborectalis muscle (MEP), cervix and vastus lateralis muscle and mode of delivery. Inclusion criteria were nulliparity, a singleton pregnancy and good knowledge of the Dutch language. Exclusion criteria were an age under 18 years, a history of pelvic organ prolapse surgery, incontinence surgery or previous surgery to the uterus, connective tissue disease and an inability to perform a maximum Valsalva maneuverer because of cardiac or pulmonary disease. All women gave informed consent, the study was approved by the Institutional Human Research Ethics Committee (reference 14/482) and registered in the Dutch Trial Register "Nederlands Trial Register (NTR)" (NTR 4882).

The participants were invited for a transperineal 3D/4D ultrasound of the pelvic floor, an endovaginal ultrasound of the cervix and an ultrasound of the vastus lateralis muscle at 12 weeks' gestation. Since the previously demonstrated association between MEP and mode of delivery was only demonstrated early in pregnancy, we opted for measuring at 12 weeks .[20] Two of the participating hospitals used a GE Voluson 730 Expert ultrasound system (GE Healthcare, Zipf, Austria) with a RAB 4-8 MHz curved array 3D/4D ultrasound transducer and a RIC 4-9 MHz endovaginal ultrasound transducer. At the third hospital, the portable Voluson *i* (GE Healthcare, Zipf, Austria) and at the fourth hospital the Voluson E8 (GE Healthcare, Zipf, Austria) were used. The intensity values were kept constant (gain 15, power 100, Harmonics mid, contrast 8, grey map 4, persistence 8, and enhance 3) in order to compare the echogenicity. The volume datasets of the pelvic floor with a fixed volume angle of 0.8 were obtained at rest, at contraction and on Valsalva maneuverer with the women in supine position and with an empty bladder. The cervix was displayed with the use of an endovaginal probe with the programmed cervix intensity values at gain -4, power 100, Harmonics mid. The right vastus lateralis muscle was imaged at 10cm above the patella with the leg at rest with the curved array transducer and the same intensity values as the cervix. All examinations at the UMC Utrecht, St Antonius hospital and Radboud UMC were performed by a single trained observer (in UMC Utrecht and St Antonius hospital, by the first author), in the Reinier de Graaf hospital by two trained observers.

Based on our previous study a difference in MEP of 19 points between the normal vaginal delivery group and women who had a CS due to FTP was expected. In order to demonstrate this difference at a significance level of 0.05 and a power of 0.8, 13 patients were required in each group. In literature the number of CS due to FTP ranges between 5-9%,[2,8] thus an initial 260 participants (calculating worst case scenario 5%) were required. If 15% were lost to follow-up a total of 306 patients was required for this study.

After storage on hard disk, offline analysis was performed using the 4D View 7.0 (GE Medical Systems Kretztechnik, Zipf, Austria) (for MEP and puborectalis muscle area (PMA)) and Matlab® R2010a (MathWorks, Natick, MA) (for MEP, PMA, cervix and vastus lateralis muscle) software. The plane of minimal hiatal dimension of the puborectalis muscle in axial position was selected and exported for the automatic analysis of the MEP and area (PMA) and hiatal dimensions of the puborectalis muscle.[18] Echogenicity is based on the greyscale image in which the value for each pixel can vary between 0 (black) and 255 (white).

Since the cervical canal consist mainly of mucus and water, and the acoustic impedance of mucus and water is smaller than the acoustic impedance of soft tissue, we only investigated the part of the cervix in direct contact with the probe. We selected the total representative part of the cervix and artefacts at the sides of the external os and the internal os in the image were ignored during the delineation (Figure 1a).

The representative part of the longitudinal image of the vastus lateralis muscle was selected as shown in figure 1b (Figure 1b).

Obstetric data were abstracted from the patient record file and mode of delivery was classified into five categories: spontaneous vaginal delivery, vacuum extraction (ventouse), elective CS, CS due to FTP and CS due to FD.

Statistical analysis was performed using the statistical package IBM SPSS statistics 20.0 for Windows. The MEP and PMA of different delivery modes were compared with an ANOVA analysis with Tukey's posthoc test when applicable. The factor used was: mode of delivery; the dependent list was: MEP at rest/contraction/Valsalva, hiatal area at rest/contraction/Valsalva, hiatal transverse diameter at rest/contraction/Valsalva, strain, echogenicity of the cervix and echogenicity of the vastus lateralis muscle.

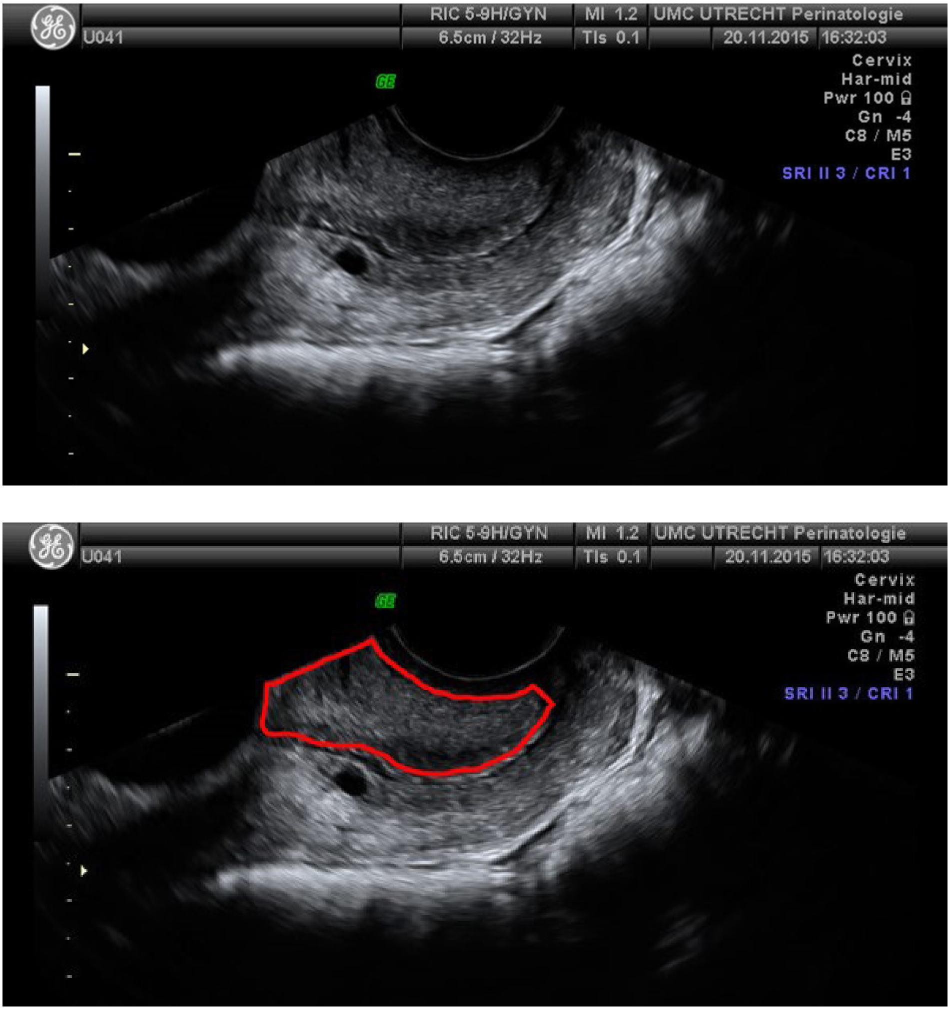
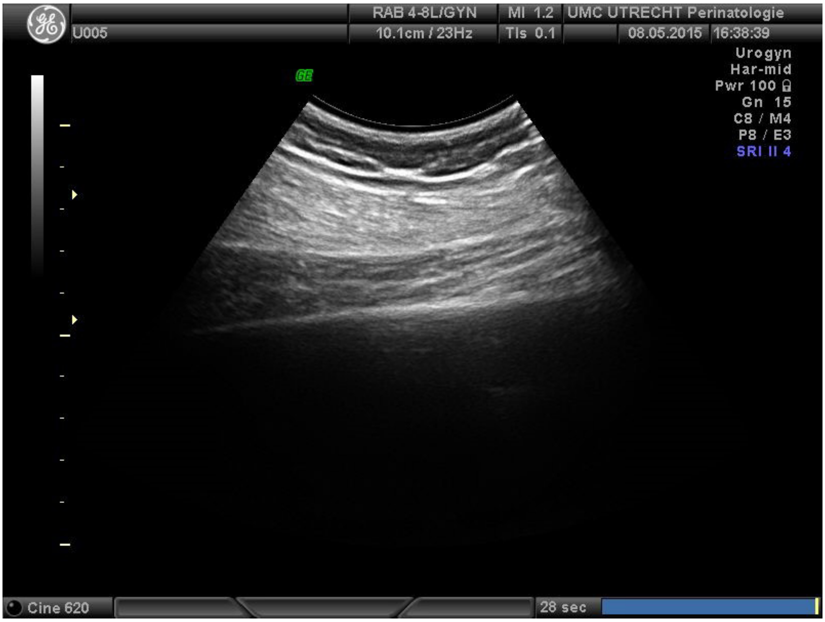


Figure 1a. Delineation of the anterior lip of the cervix.



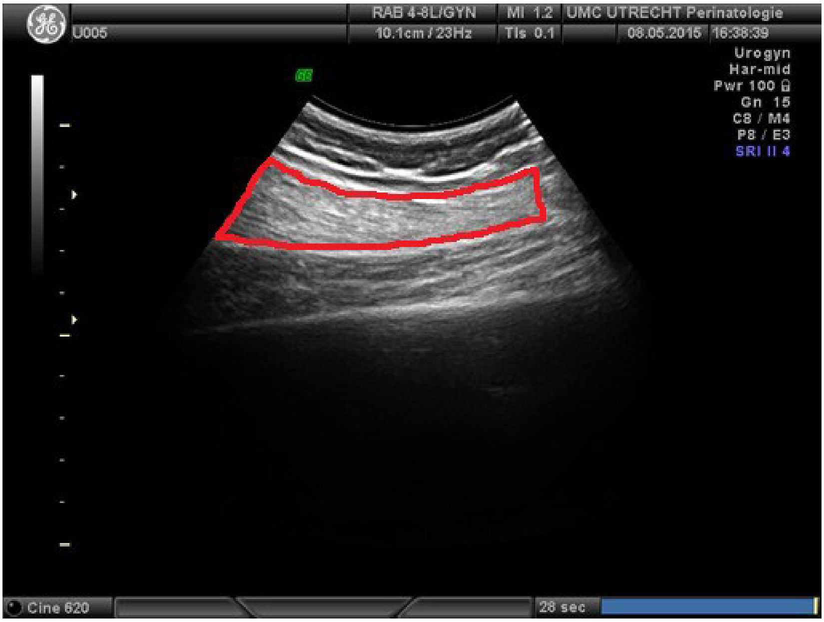


Figure 1b. Delineation of the representative part of the vastus lateralis muscle.

Results

Between March 2015 and January 2017, 306 nulliparous women with a singleton pregnancy were included in the 4 participating hospitals. Two out of the 4 hospitals (UMC Utrecht and St Antonius hospital), accounted for 274 out of the 306 inclusions. All ultrasound scans in these 2 hospitals were performed by only one trained observer (KW), using the same ultrasound machine (Voluson 730). Within these 274 inclusions we already obtained ultrasound data of 24 women who had a CS due to FTP, enough for our sample size. The process of developing a conversion factor to account for differences between the ultrasound machines (despite the same manual settings) is very time consuming. Since we already reached our sample size of women with FTP CS, we decided not to develop such a factor for the small datasets (22 and 10 women) from the two other hospitals. In this study we therefore only present the datasets of the UMC Utrecht and St Antonius hospital (Figure 2).[23,24]

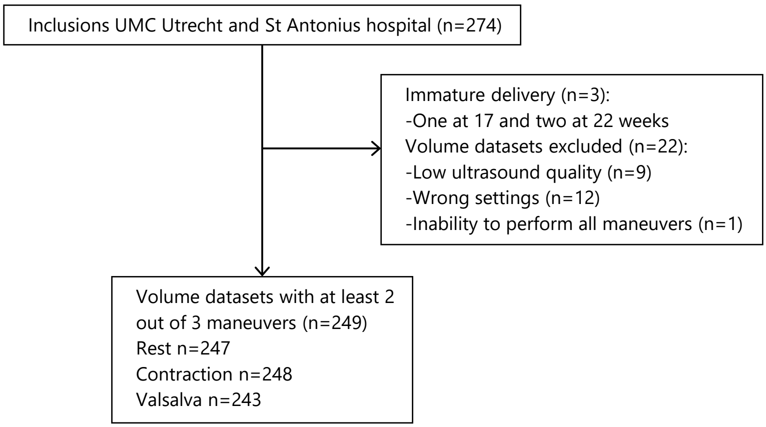


Figure 2. Flowchart illustrating the number of volume datasets obtained and analyzed.

Patient characteristics are shown in Table 1. Differences in the characteristics between the different mode of delivery groups were found in; gestational age at delivery (lower outcome in elective CS (37.7 weeks), p<0.01) and birth weight (BW) (higher BW in CS due to FTP (3694 gram) and lower in elective CS (2964 gram) and CS due to FD (3043 gram), p<0.01).

Table 1. Patient characteristics

Age, mean (SD)	30.5 (4.1)
BMI, median (range)	22.9 (15.8-41.5)
GA at visit, median (range)	12.7 (8.6-14.9)
GA at delivery, median (range)	40.1 (26.3-42.4)
Type of delivery, n (%)	
Spontaneous vaginal	166 (66.7)
Ventouse	34 (13.7)
Elective CS	14 (5.6)
CS due to FTP	24 (9.6)
CS due to FD	11 (4.4)
Birth weight, mean (SD)	3306.5 (584.0)
Sexe; girl, n (%)	129 (51.8)

GA=Gestational age

Mean echogenicity of the puborectalis muscle

The mean MEP at rest, contraction and Valsalva was respectively 112, 106 and 111. Although the MEP during contraction is lower in the FTP group, this was not statistically significant different (Figure 3). The MEP at rest and at Valsalva maneuver was also not statistically different between the different modes of delivery.

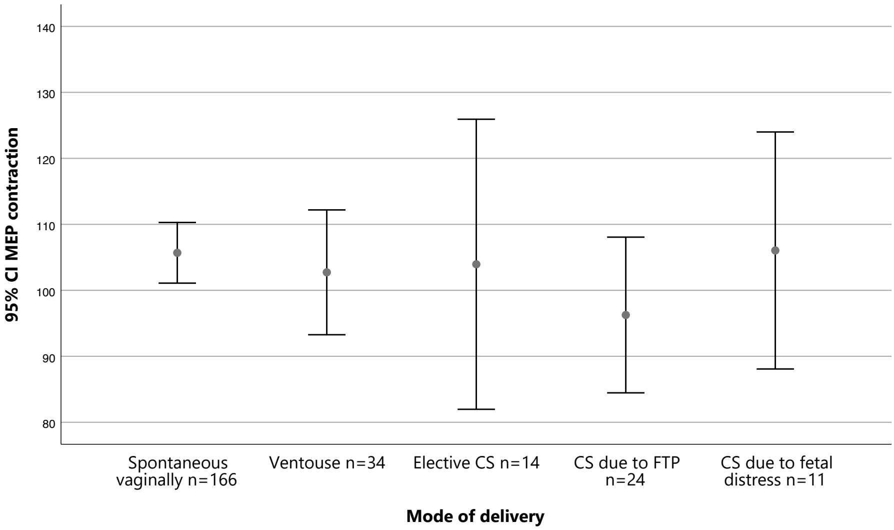


Figure 3. Distribution of the mean MEP at contraction at 12 weeks' gestation and the 95%-confidence intervals.

Table 2. Hiatal dimensions

	Total Mean (SD) n=249	Spontaneous vaginally Mean (SD) n=166
Hiatal area rest (cm^2)	12.83 (2.45) n=247	13.01 (2.45) n=165
Hiatal area contraction (cm^2)	10.78 (1.98) n=248	10.91 (2.00) n=165
Hiatal area Valsalva (cm^2)	14.29 (3.31) n=243	14.54 (3.27) n=162
Transverse rest (cm)	3.97 (0.40) n=247	4.01 (0.40) n=165
Transverse contraction (cm)	3.73 (0.37) n=248	3.76 (0.36) n=165
Transverse Valsalva (cm)	4.10 (0.42) n=243	4.14 (0.43) n=162
Strain (decrease in hiatal area rest-contraction) (%)	14.66 (12.82) n=246	14.73 (12.88) n=164

AP=anteroposterior diameter

Hiatal dimensions

The ANOVA showed no statistically significant differences between the hiatal dimensions or the strain of the hiatal area and modes of delivery (Table 2).

Echogenicity of the cervix

The mean echogenicity of the cervix in relation to the mode of delivery is shown in figure 4. The ANOVA test showed no statistically significant differences.

Echogenicity of the vastus lateralis muscle

The mean echogenicity of the vastus lateralis muscle in relation to the mode of delivery is shown in figure 5. The ANOVA test showed no statistically significant differences.

Ventouse Mean (SD) n=34	Elective CS Mean (SD) n=14	CS due to FTP Mean (SD) n=24	CS due to FD Mean (SD) n=11
12.44 (2.20) n=34	11.91 (1.54) n=14	12.42 (2.76) n=23	13.41 (3.24) n=11
10.40 (1.91) n=34	10.40 (1.61) n=14	10.42 (2.39) n=24	11.23 (1.27) n=11
13.36 (2.93) n=32	12.99 (2.93) n=14	13.67 (3.59) n=24	16.30 (3.62) n=11
3.89 (0.36) n=34	3.86 (0.25) n=14	3.91 (0.52) n=23	3.91 (0.34) n=11
3.65 (0.37) n=34	3.69 (0.32) n=14	3.64 (0.53) n=24	3.84 (0.19) n=11
3.99 (0.42) n=32	3.96 (0.38) n=14	4.01 (0.46) n=24	4.16 (0.31) n=11
15.95 (10.34) n=34	12.53 (9.55) n=14	14.57 (14.61) n=23	12.49 (19.02) n=11

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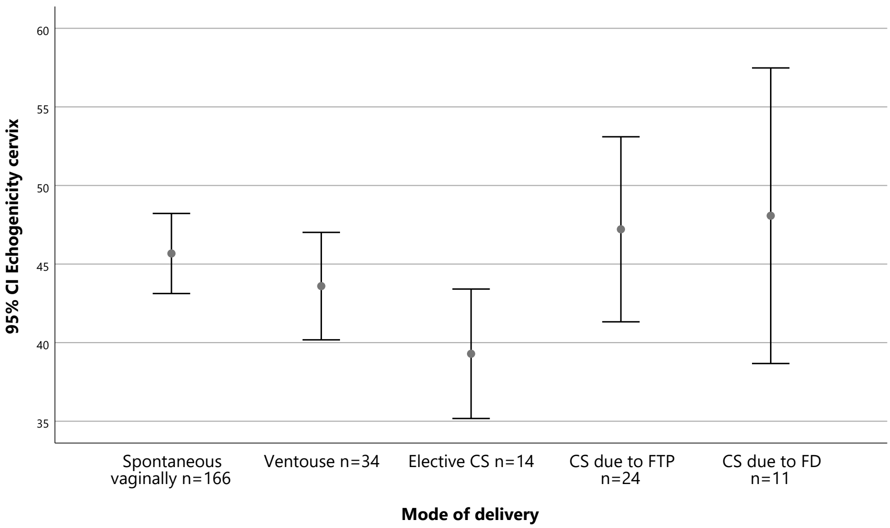


Figure 4. Distribution of the mean echogenicity of the cervix at 12 weeks' gestation and the 95%-confidence intervals.

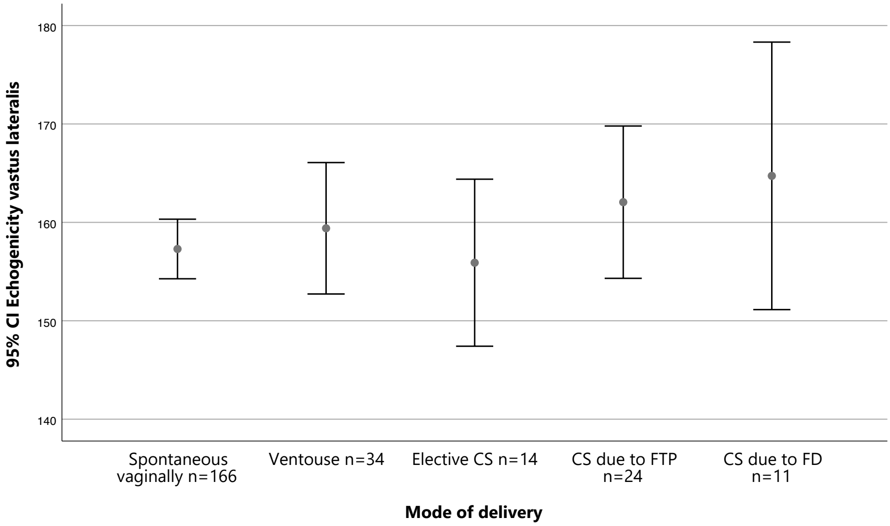


Figure 5. Distribution of the mean echogenicity of the vastus lateralis muscle at 12 weeks' gestation and the 95%-confidence intervals.

Discussion

In this study we were unable to confirm our previous finding that the MEP and hiatal transverse diameter and area at 12 weeks gestation are significantly associated with the mode of delivery.[19,20] In addition, we could not demonstrate a significant association between the echogenicity of the cervix and vastus lateralis muscle and mode of delivery. Furthermore, no significant differences between the levator hiatal dimensions and the modes of delivery are found. What we did notice however, was a mean 20 points lower MEP in our database as compared to the previous study, despite the use of the same ultrasound equipment.

Until now, only one single center study demonstrated a possible relation between echogenicity of the puborectalis muscle early in pregnancy and mode of delivery.[20] Furthermore, the association between MEP, transverse hiatal diameter and area was only found during a muscle contraction.[19,20] Possible explanations for the different outcome of this study are considered. Differences in the decision-making process towards a CS in the different hospitals in this multicenter study might explain why we could not confirm our previous findings. Therefore, a sub analysis of the UMC Utrecht population of this study was performed since the previous study was performed only at the UMC Utrecht. However, this analysis also showed no difference in the MEP between the different modes of delivery in the UMC Utrecht population. Another possible explanation is a difference in the delineation of the puborectalis muscle by hand between the observers in the previous and present study. We could reject this idea because we showed an almost perfect inter-observer reliability of the MEP and area measurements before.[18] We also delineated the images of the puborectalis muscle of the women with a CS due to FTP of the previous study again and found no differences between the original data and re-analyzed data of the previous study.[20] Another possible explanation is a difference in the instruction to do a proper maximal muscle contraction maneuver. We therefore compared the strain, expressed as difference in hiatal area between rest and contraction of the total group, to the strain of the women in the previous studies and found no differences.[19,20]

In this study, the general MEP was lower compared to the study of Grob et al. because of a higher depth in all ultrasound images of the current study.[20] A higher depth, and especially because the depth exceeds 7.4cm (contrary to the previous study in which the depth in all ultrasound images was <7.4cm), resulted in a lower echogenicity because of changes in beamforming of the ultrasound machine. Since a lower MEP was found in all groups, the mean between the groups is comparable.

If there is an association between the echogenicity of the cervix and a CS due to FTP, we expected a lower echogenicity in this group. A lower echogenicity reflects less ECM and less collagen. With a predominance of muscle cells, the process of ripening and dilatation of the cervix, as a resultant of weakening of the collagen matrix, is expected to slow down. With failure to progress as a consequence. We were unable to confirm this hypothesis. The values of the echogenicity of the cervix were almost comparable to the echogenicity values of the cervix in pregnant women in the second trimester of the study of Kuwata et al. who showed values of 30.0 to 32.9 in the region of interest of the anterior lip of the cervix (in the mid-section of the anterior and posterior cervical boundaries from a 7x7 mm2 area containing 29x29 pixels).[26] But since they used an ultrasound machine of a different brand, these values are just an indication.

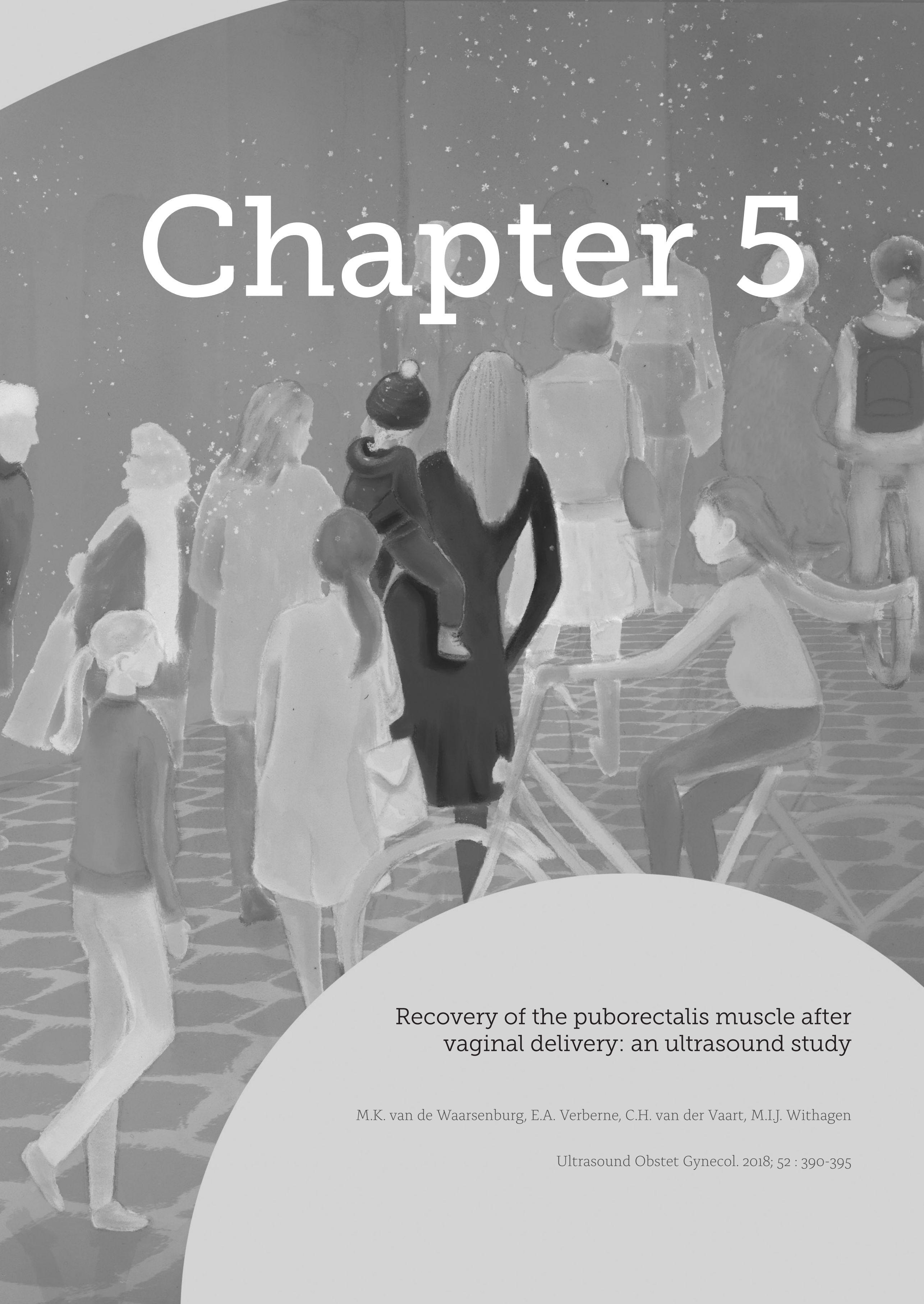
Since the effect of pregnancy on muscle tissue composition could differ between the pelvic floor and non-pelvic floor muscles, as seen in the tibialis muscle of rats, we hypothesize that there is no association between the echogenicity of the vastus lateralis muscle and the modes of delivery.[21,22] We found no association between the echogenicity of the vastus lateralis muscle and the modes of delivery. But since we were unable to show an association between the echogenicity of the puborectalis muscle and the cervix and a CS due to FTP and the echogenicity before pregnancy is unknown, we could not confirm or reject our hypothesis.

In conclusion: In a second, independent multicenter dataset we were unable to confirm our previous finding that the hiatal dimensions and MEP on contraction were statistically significant associated with the mode of delivery.

References

1. Kwee A, Elferink-Stinkens PM, Reuwer PJ, Bruinse HW. Trends in obstetric interventions in the Dutch obstetrical care system in the period 1993-2002. *Eur J Obstet Gynecol Reprod Biol* 2007; 132 : 70-75.
2. Reuwer PJ, Bruinse HW, Franx A. *Proactive support of labour*. Cambridge university press, 2015.
3. Kozinszky Z, Orvos H, Zoboki T, Katona M, Wayda K, Pal A, Kovacs L. Risk factors for cesarean section of primiparous women aged over 35 years. *Acta Obstet Gynecol Scand* 2002; 81 : 313-316.
4. Usha Kiran TS, Hemmadi S, Bethel J, Evans J. Outcome of pregnancy in a woman with an increased body mass index. *BJOG* 2005; 112 : 768-772.
5. Fitzpatrick M, McQuillan K, O'Herlihy C. Influence of persistent occiput posterior position on delivery outcome. *Obstet Gynecol* 2001; 98 : 1027-1031.
6. Morris JM, Thompson K, Smithey J, Gaffney G, Cooke I, Chamberlain P, Hope P, Altman D, MacKenzie IZ. The usefulness of ultrasound assessment of amniotic fluid in predicting adverse outcome in prolonged pregnancy: a prospective blinded observational study. *BJOG* 2003; 110 : 989-994.
7. Saunders N, Amis S, Marsh M. The prognostic value of fetal ultrasonography before induction of labour. *Br J Obstet Gynaecol* 1992; 99 : 903-906.
8. Schuit E, Kwee A, Westerhuis ME, Van Dessel HJ, Graziosi GC, Van Lith JM, Nijhuis JG, Oei SG, Oosterbaan HP, Schuitemaker NW, Wouters MG, Visser GH, Mol BW, Moons KG, Groenwold RH. A clinical prediction model to assess the risk of operative delivery. *BJOG* 2012; 119 : 915-923.
9. Peregrine E, O'Brien P, Omar R, Jauniaux E. Clinical and ultrasound parameters to predict the risk of cesarean delivery after induction of labor. *Obstet Gynecol* 2006; 107 : 227-233.
10. Chen G, Uryasev S, Young TK. On prediction of the cesarean delivery risk in a large private practice. *Am J Obstet Gynecol* 2004; 191 : 616-24; discussion 624-5.
11. Harper DM, Johnson CA, Harper WH, Liese BS. Prenatal predictors of cesarean section due to labor arrest. *Arch Gynecol Obstet* 1995; 256 : 67-74.
12. Hueston WJ. Development of a cesarean delivery risk score. *Obstet Gynecol* 1994; 84 : 965-968.
13. Roman H, Blondel B, Breart G, Goffinet F. Do risk factors for elective cesarean section differ from those of cesarean section during labor in low risk pregnancies? *J Perinat Med* 2008; 36 : 297-305.
14. Seshadri L, Mukherjee B. A predictive model for cesarean section in low risk pregnancies. *Int J Gynaecol Obstet* 2005; 89 : 94-98.
15. Lien KC, Mooney B, DeLancey JO, Ashton-Miller JA. Levator ani muscle stretch induced by simulated vaginal birth. *Obstet Gynecol* 2004; 103 : 31-40.
16. Mayans D, Cartwright MS, Walker FO. Neuromuscular ultrasonography: quantifying muscle and nerve measurements. *Phys Med Rehabil Clin N Am* 2012; 23 : 133-48, xii.
17. Pillen S, Verrips A, van Alfen N, Arts IM, Sie LT, Zwarts MJ. Quantitative skeletal muscle ultrasound: diagnostic value in childhood neuromuscular disease. *Neuromuscul Disord* 2007; 17 : 509-516.
18. Grob AT, Veen AA, Schweitzer KJ, Withagen MI, van Veelen GA, van der Vaart CH. Measuring echogenicity and area of the puborectalis muscle: method and reliability. *Ultrasound Obstet Gynecol* 2014; 44 : 481-485.
19. Van Veelen GA, Schweitzer KJ, van Hoogenhuijze NE, van der Vaart CH. Association between levator hiatal dimensions on ultrasound during first pregnancy and mode of delivery. *Ultrasound Obstet Gynecol* 2015; 45 : 333-338.
20. Grob AT, Withagen MI, van de Waarsenburg MK, Schweitzer KJ, van der Vaart CH. Association of First-Trimester Echogenicity of the Puborectalis Muscle With Mode of Delivery. *Obstet Gynecol* 2016; 127 : 1021-1026.

21. Alperin M, Kaddis T, Pichika R, Esparza MC, Lieber RL. Pregnancy-induced adaptations in intramuscular extracellular matrix of rat pelvic floor muscles. *Am J Obstet Gynecol* 2016; 215 : 210.e1-210.e7.
22. Alperin M, Lawley DM, Esparza MC, Lieber RL. Pregnancy-induced adaptations in the intrinsic structure of rat pelvic floor muscles. *Am J Obstet Gynecol* 2015; 213 : 191.e1-191.e7.
23. Pillen S, van Alfen N. Skeletal muscle ultrasound. *Neurol Res* 2011; 33 : 1016-1024.
24. Van de Waarsenburg MK, van der Vaart CH, Withagen MIJ. Structural changes in the puborectalis muscle after vaginal delivery. *Ultrasound Obstet Gynecol* 2018; .
25. Van Veelen GA, Schweitzer KJ, van der Vaart CH. Ultrasound imaging of the pelvic floor: changes in anatomy during and after first pregnancy. *Ultrasound Obstet Gynecol* 2014; 44 : 476-480.
26. Kuwata T, Matsubara S, Taniguchi N, Ohkuchi A, Ohkusa T, Suzuki M. A novel method for evaluating uterine cervical consistency using vaginal ultrasound gray-level histogram. *J Perinat Med* 2010; 38 : 491-494.



Chapter 5

Recovery of the puborectalis muscle after vaginal delivery: an ultrasound study

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Abstract

Objective: To assess change in levator hiatus dimensions between pregnancy and different timepoints after vaginal delivery, and map recovery of the hiatus in order to contribute to secondary prevention of symptoms of pelvic floor disorders.

Methods: Twenty nulliparous women with a singleton pregnancy received ultrasound assessment of the pelvic floor at rest, on PFMC and on Valsalva maneuver at 12 weeks' gestation and at 1 day and 1, 2, 3, 4, 6, 12, 18 and 24 weeks after vaginal delivery. Dimensions of the levator hiatus were measured and contractility and distensibility were calculated. Wilcoxon Signed Ranks Tests were used to compare each postpartum value with 12 weeks' gestation.

Results: One day, 1 and 2 weeks after vaginal delivery hiatal areas at rest, on PFMC and on Valsalva maneuver were significantly increased compared to 12 weeks' gestation. From three weeks postpartum on, hiatal areas at rest and on PFMC, as well as contractility from six weeks on were comparable to 12 weeks' gestation, however on Valsalva there remains a significant difference until 24 weeks' after delivery. Moreover, distensibility was still increased at 24 weeks postpartum compared to 12 weeks' gestation.

Conclusion: The puborectalis muscle has the ability to recover anatomically from first vaginal delivery and most recovery occurs during the first three weeks after delivery. Stretch of the puborectalis muscle, as reflected by distensibility, persisted 24 weeks after that first vaginal delivery. The data provide a better understanding of the early "normal" regeneration process and we hypothesize that the first three weeks postpartum is the best window to start secondary prevention.

Introduction

Pelvic floor disorders such as pelvic organ prolapse and urinary incontinence are highly prevalent conditions with a negative impact on quality of life.[1] Vaginal delivery is an important risk factor for these conditions.[2,3] During vaginal delivery, the pelvic floor muscles are stretched and compressed against the pelvic sidewall, which can induce injuries. Computer modeling based on magnetic resonance imaging (MRI) has shown that, during passage of the fetal head, the levator ani muscle stretches to at least twice its initial length.[4] Several clinical studies have found an increase in area of the levator hiatus after vaginal delivery, indicating function loss possibly by tissue trauma.[5-7] As the levator ani muscle plays an important role in pelvic organ support, it is not surprising that levator avulsion and large hiatal area have been associated with prolapse symptoms and stress urinary incontinence.[7-10] However, not all vaginally parous women develop pelvic floor disorders later in life. It is still not fully understood which women are at increased risk of experiencing symptoms. This may be related to the extent of the damage that occurs during delivery and/or differences in the recovery process.

Little is known about normal recovery of the pelvic floor in the first weeks after vaginal delivery. In particular, there is a paucity of longitudinal data on this recovery.

A better understanding of the recovery of the pelvic floor after vaginal delivery is of importance when considering early interventions to improve this recovery. As we know that primary prevention of symptomatical pelvic floor disorders is difficult, there is a focus on secondary prevention with stem cells and its culture medium.[11-13]

This study was designed to evaluate levator hiatal dimensions using 3D/4D transperineal ultrasound at several timepoints after vaginal delivery, from 1 day up to and including 24 weeks postpartum, and to compare these with dimensions in early pregnancy in order to provide a better understanding early “normal”, recovery of the puborectalis muscle.

Methods

This study was part of a prospective multicenter cohort study on the association between mean echogenicity of the puborectalis muscle and mode of delivery. Three hundred six nulliparous women with a singleton pregnancy and good knowledge of the Dutch language were included in the original study. Exclusion criteria were a history of pelvic organ prolapse surgery, incontinence surgery, surgery in the uterus wherefore they had to deliver by Cesarean, connective tissue disease or an inability to perform a maximum Valsalva maneuver because of cardiac or pulmonary disease. The study was approved by the Institutional Human Research Ethics Committee (reference 14/482) and all women gave informed consent to participate

All participants underwent an ultrasound assessment of the pelvic floor at 12 weeks' gestation. Patients were also asked to participate in a follow-up study and the first 20 patients that gave informed consent and delivered vaginally were included in this sub study. These 20 women received ultrasound follow-up at 1 day and 1, 2, 3, 4, 6, 12, 18 and 24 weeks after vaginal delivery and gave informed consent. The ultrasound device used was either the GE Voluson 730 Expert system (GE Healthcare) or the portable Voluson *i* (GE Healthcare), as women had the choice to have the ultrasounds made at our clinic or in their home. At each follow-up timepoint, transperineal ultrasound was performed at rest, on maximum pelvic floor muscle contraction (PFMC) and on maximum Valsalva maneuver, with the participant in supine position and with an empty bladder. Each maneuver was recorded at least three times and the obtained volume imaging datasets were stored on a hard disk. Offline analysis was performed using 4D View 7.0 (GE Medical Systems Kretztechnik, Zipf, Austria) software. The plane of minimal hiatal dimensions in axial position was selected and dimensions of the levator hiatus (anteroposterior (AP) diameter, transverse diameter and area) were measured at rest, on PFMC and on Valsalva maneuver, as previously described by Dietz *et al*.[14] In short, the AP diameter of the levator hiatus was defined as the distance from the inferior border of the symphysis pubis to the inner border of the puborectalis muscle. The transverse diameter of the levator hiatus was defined as the longest distance between two inner sides of the puborectalis muscle, perpendicular the AP diameter. The area of the levator hiatus was measured as the area bordered by the puborectalis muscle, the symphysis pubis and the inferior pubic ramus (Figure 1).

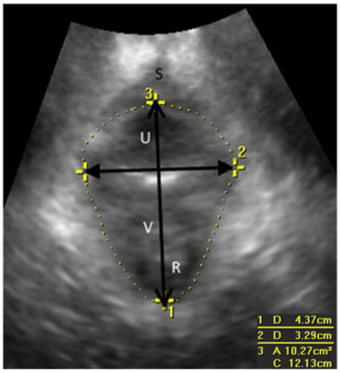


Figure 1. Measurement of levator hiatal dimensions in the axial plane at rest. Vertical arrow represents anteroposterior diameter (D) of the levator hiatus (1 D), horizontal arrow represents transverse diameter of levator hiatus (2 D) and dotted line is area (A) of levator hiatus. C=circumference; R=rectum; S=symphysis pubis; U=urethra; V=vagina.

5

Contractility and distensibility were calculated by subtracting hiatal dimensions during PFMC from hiatal dimensions at rest and hiatal dimensions at rest from hiatal dimensions during Valsalva, respectively.[15] The observer was blinded for all clinical data, but not for the time at which the ultrasound was performed. The presence of an avulsion of the puborectalis muscle was defined in line with the international accepted definition presented by Dietz *et al.* in 2007 and 2011 and performed by an experienced observer (MW).[16,17]

Demographic (age, ethnicity, body mass index) and delivery (gestational age, mode, perineum damage, birth weight) data were obtained from the women's clinical files. Considering the large number of ultrasound follow up moments and time investment, we decided to include 20 women as a pilot sample.

Statistical analysis was performed using SPSS for Windows version 22.0 (IBM Corp., Armonk, NY, USA). Levator hiatal area and contractibility/distensibility were described as medians (range). Hiatal dimensions at each follow-up were compared with hiatal dimensions at 12 weeks' gestation, using Wilcoxon Signed Ranks Tests. $P<0.05$ was considered significant.

Results

Of the 200 scheduled transperineal ultrasound examinations, 193 were performed on the 20 participants at 10 timepoints, the first one at approximately 12 (range 9.7 – 14.0 weeks) weeks' gestation and nine at different follow-up time after vaginal delivery (Figure 2).

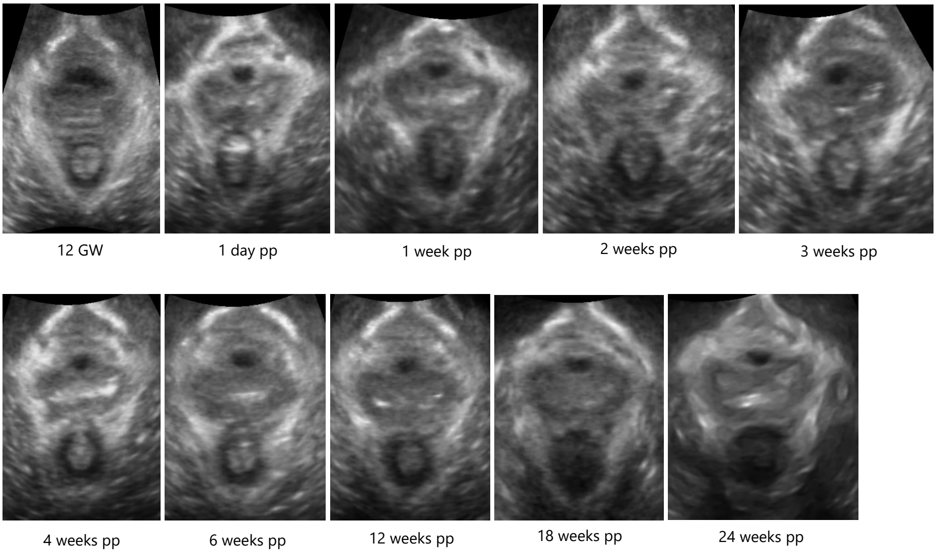


Figure 2. Transperineal ultrasound image of pelvic floor at rest in same women at 12 gestational weeks (GW), as well as 1 day and 1, 2, 3, 4, 6, 12, 18 and 24 weeks postpartum (pp).

Seven ultrasound examinations were not performed at all, and three volume datasets for the levator hiatal measurements and four for the levator ani avulsions were excluded later due to low ultrasound quality (Figure 3).

Median postpartum time of ultrasound follow up as well as other patient characteristics are described in Table 1.

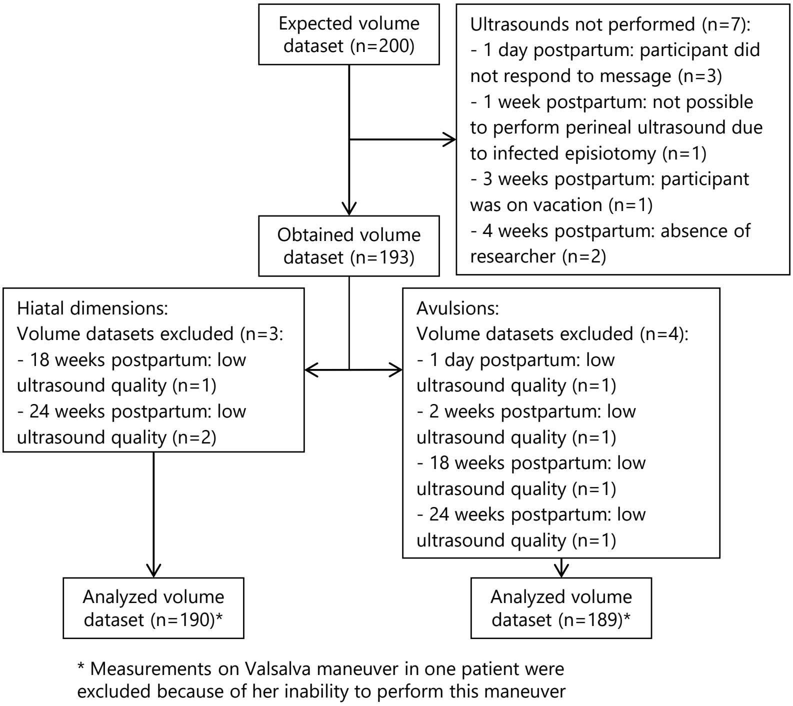


Figure 3. Diagram illustrating the number of volume datasets obtained and analyzed of the 20 participants

Levator hiatal dimensions

Median areas of the levator hiatus at 12 weeks' gestation and at 1 day and 1, 2, 3, 4, 6, 12, 18 and 24 weeks after vaginal delivery are shown in Figure 4. One day after vaginal delivery hiatal areas at rest, on PFMC and on Valsalva maneuver were significantly increased compared to 12 weeks' gestation ($p<0.005$). Hiatal areas at rest and on PFMC declined during the first 2 weeks postpartum, but were still significantly larger than at 12 weeks' gestation ($p<0.03$ for rest and $p<0.002$ for PFMC). After the first 2 weeks postpartum, hiatal areas at rest and on PFMC were no longer significantly different from 12 weeks' gestation, with the exception of hiatal area at rest at the 18th week postpartum ($p=0.03$) and hiatal area on PFMC at 6 weeks postpartum ($p=0.02$). Hiatal area on Valsalva maneuver remained significantly increased compared to 12 weeks' gestation up to and including 18 weeks postpartum ($p<0.002$). At 24 weeks postpartum, hiatal area on Valsalva maneuver was still increased, but not significantly different from 12 weeks' gestation ($p=0.08$).

Table 1. Maternal and delivery characteristics of 20 women included in the study

Parameter	Value
Age (years)	32 (27 – 41)
Ethnicity	
Caucasian	19 (95)
Other	1 (5)
Body mass index at 12 weeks pregnancy	23.4 (19.2 – 37.6)
Gestational age at first ultrasound (wk)	11.9 (9.7 – 14.0)
Gestational age at delivery (wk)	40.1 (31.0 – 41.4)
Delivery mode	
Normal vaginal delivery	15 (75)
Assisted vaginal delivery (vacuum)	5 (25)
Perineum	
Undamaged	5 (25)
Perineal tear	6 (30)
Episiotomy	9 (45)
Neonatal birth weight (g)	3432 (1750 – 4004)

Data are given as median (range) or *n* (%). BMI=body mass index; GA=gestational age

Median contractility and distensibility of the levator hiatus at 12 weeks' gestation and at 1 day and 1, 2, 3, 4, 6, 12, 18 and 24 weeks after vaginal delivery are presented in Figure 5. Compared with measurements at 12 weeks' gestation, contractility after significantly lower ($p<0.04$) up to and including the sixth week postpartum, with the exception of 1 day and 2 weeks postpartum. Distensibility increased significantly from 2 weeks after vaginal delivery up to the last measurement at 24 weeks postpartum, compared with measurements at 12 weeks.

Hiatal dimensions at rest, on PFMC, and on Valsalva maneuver at all ultrasound examinations, along with significance levels compared with measurements at 12 GW, in appendix.

Levator avulsion

Of the 20 patients, 12 did not have any type of avulsion. Two women had a partial left and one had a partial bilateral avulsion. Two women had a complete right avulsion and three women had complete bilateral avulsions. The regeneration pattern of the women with a complete bilateral avulsion is shown is Figure 6.

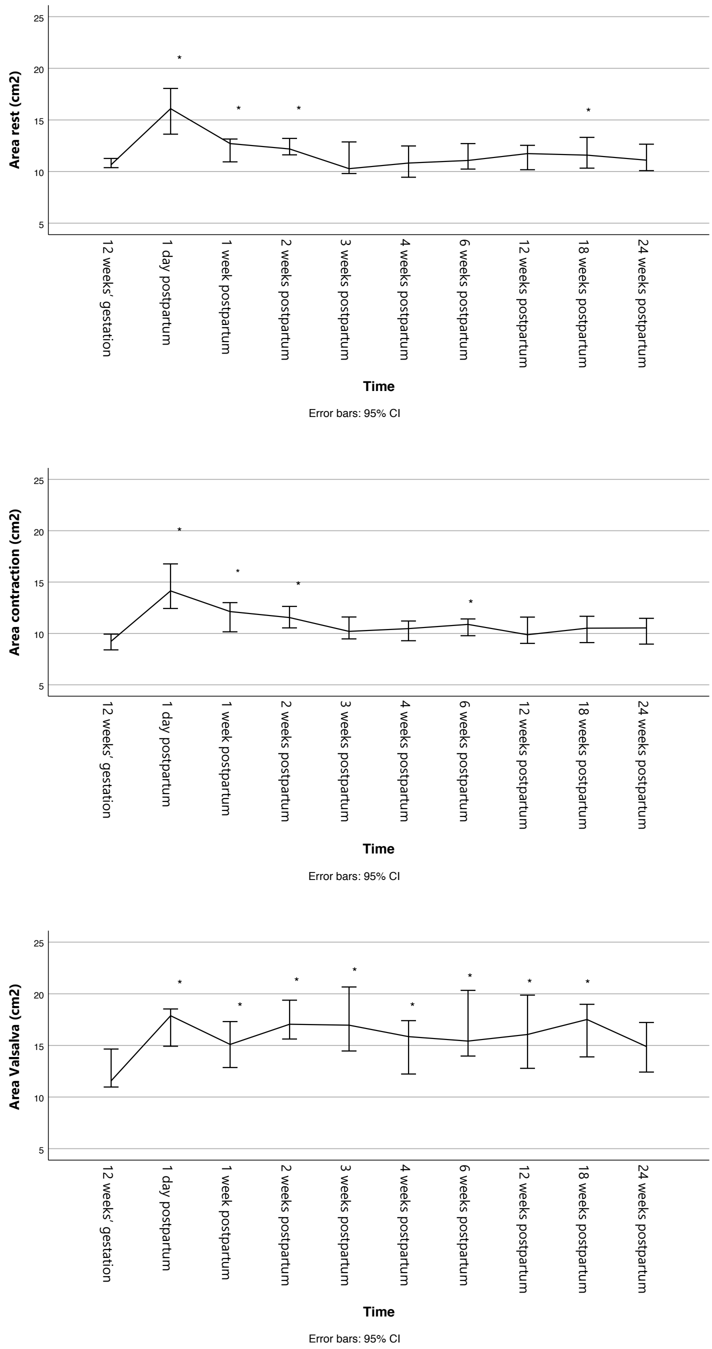
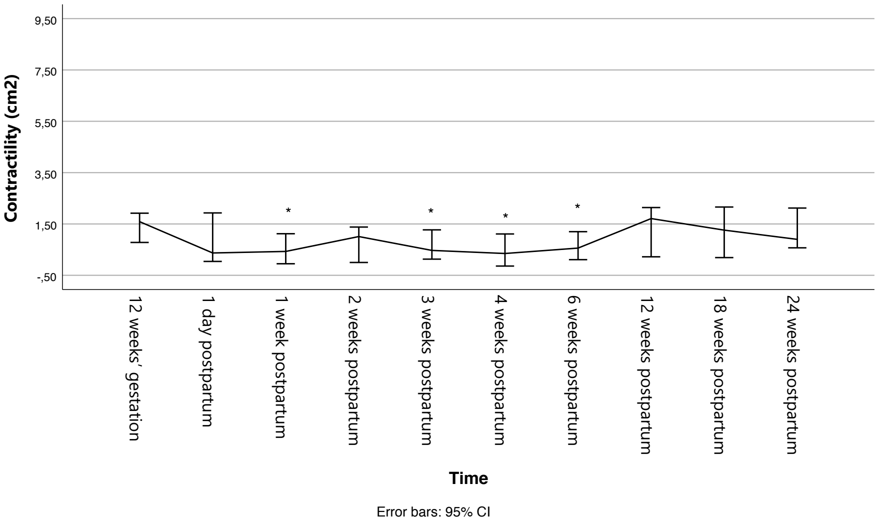


Figure 4. Median area of the levator hiatus (LH) at rest, on PFMC and on maximum Valsalva maneuver at 12 GW and at 1 day and 1, 2, 3, 4, 6, 12, 18 and 24 weeks postpartum (pp) after vaginal delivery. Bars are 95%-CI. * Significant difference from measured at 12 GW.



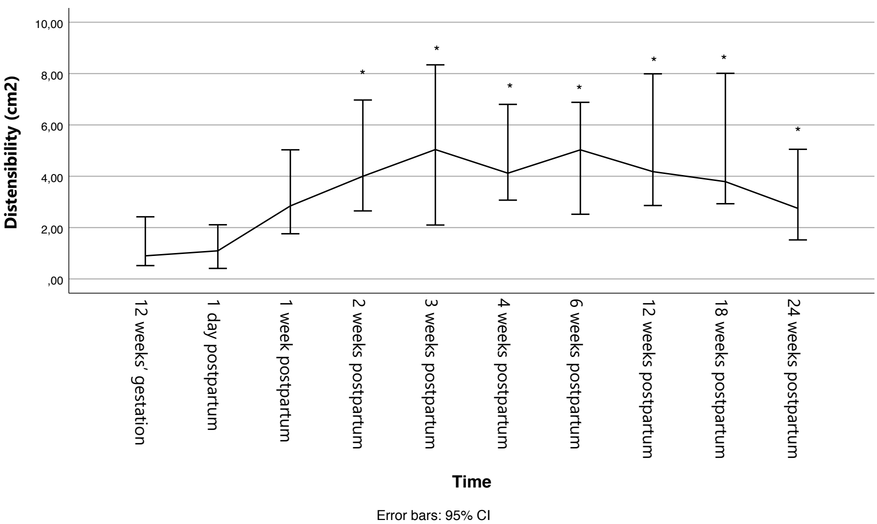
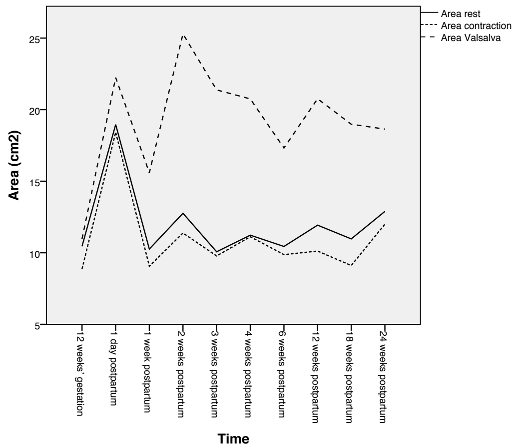


Figure 5. Median contractility (difference between levator hiatal (LH) area at rest and on PFMC) and distensibility (difference between LH area on Valsalva maneuver and at rest) of puborectalis muscle at 12 GW and at 1 day and 1, 2, 3, 4, 6, 12, 18 and 24 weeks postpartum. Bars are 95%-CI. *Significant difference from measurement at 12 GW.

A



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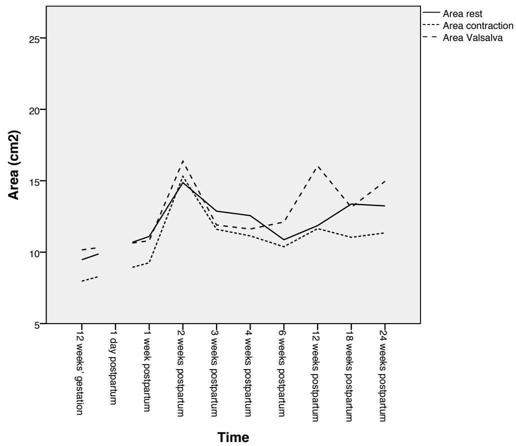

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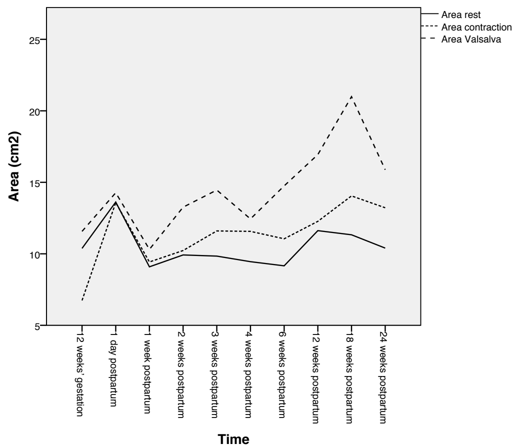


Figure 6. Levator hiatal area at rest, on maximum PFMC and on maximum Valsalva maneuver at 12 GW and at 1 day and 1, 2, 3, 4, 6, 12, 18 and 24 weeks postpartum (pp) in three women (A-C) with a complete bilateral avulsion. Data missing for 1 day pp in second patient (B).

Discussion

Our findings indicate that recovery to early pregnancy state of the hiatal dimensions at rest and on PFMC takes place during the first 3 weeks after vaginal delivery. On Valsalva maneuver, there seems to be a long lasting increase in levator hiatus dimensions after vaginal delivery. Contractility of the puborectalis muscle returns to early pregnancy state between 6 and 12 weeks after vaginal delivery, while distensibility of the puborectalis muscle is still increased at 24 weeks after delivery.

Strengths of our study include the longitudinal study design with nine follow up moments early postpartum (at 1 day) up to 24 weeks postpartum. The comparison between hiatal areas postpartum and those at 12 weeks' gestation is another strength, and as hiatal area increases as the pregnancy advances,[5,6,18] hiatal dimensions in the first trimester are probably closest to prepregnancy values.

Limitations include the small study population and a lack of prepregnancy values. First, a larger population would have provided more robust data. However, there were no data available from the literature with which to perform a sample size calculation. This study has to be regarded as a pilot study in which the direction and magnitude of changes that were assessed can be used for future protocol design and sample size calculations. Secondly, the population consisted of Caucasian women, with a median age of 32, and therefore the conclusions may not be applicable to other age or ethnic groups. Thirdly, one woman delivered prematurely at 31 weeks' gestation, which could possibly have led to less damage of the pelvic floor muscles, leading to an overestimation of recovery of the puborectalis muscle after vaginal delivery. However, the hiatal area of this woman was actually comparable to the group median, so we decided not to exclude this woman. Finally, our sample size limited a subgroup analysis on the effect of partial or complete levator avulsions on the recovery of the hiatal dimensions after delivery.

A study in which the levator hiatal area of six women was measured on MRI at 1 day, 1, 2, and 6 weeks and 6 months after vaginal delivery, showed that recovery took place during the first 2 weeks.[19] This is comparable to the findings in our study. Several studies found increased hiatal areas on Valsalva maneuver 6 months and 1 year after vaginal delivery.[6,15,18] In our study, the hiatal area at 24 weeks after delivery was still increased but not statistically different from 12 weeks' gestation. This finding is most likely a sample size problem.

Van Veelen *et al.* calculated contractility and distensibility at 12 weeks' gestation and 6 months after vaginal delivery, using the same method to calculate these values as we did in our study.[6] In accordance with our findings, van Veelen *et al.* found that contractility 6 months after vaginal delivery was comparable to 12 weeks' gestation, while distensibility was still significantly increased.[6]

Our finding that recovery of contractility of the puborectalis muscle takes places within 12 weeks after delivery is in accordance with studies on strain injury in skeletal muscles. In rodents, contractile function of injured tibialis anterior muscles recovers within 2 to 3 weeks.[20,21] A systematic review on rehabilitation progression and return-to-play decision making following hamstring strain injury, reported mean return-to-play time in nine studies ranging from 12 to 63 days.[22] Return-to-play time could be a reflection of recovery of contractile function.

The lack of a significant increase in distensibility during the first 3 weeks postpartum is likely due to a reluctance of women to perform maximum Valsalva maneuver shortly postpartum. The increased distensibility after vaginal delivery might be explained by permanent changes in extracellular matrix (ECM) of the levator ani muscle, as intramuscular ECM determines muscle passive mechanical properties and ability to sustain load.[23,24] However, recent studies in rats do not support this theory, as an increased ECM collagen content was found in all pelvic floor muscles during pregnancy and in only one pelvic floor muscle (coccygeus) after vaginal delivery, which indicates the presence of fibrosis.[25] Fibrosis leads to increased stiffness, which is exactly the opposite of increased distensibility. These findings, however, might not be applicable to humans, as rodents and humans differ greatly in the size of the fetus relative to the size of maternal pelvis, most likely leading to less damage of the pelvic floor during vaginal delivery in rodents.

Since the cause of increased distensibility of the levator ani muscle after vaginal delivery remains unclear, one might hypothesize that it is not vaginal delivery itself that leads to increased distensibility but in fact permanent changes induced by pregnancy. This theory is supported by the study of van Veelen, in which an increased distensibility 6 months after childbirth was observed, regardless of delivery mode.[6] However, mean distensibility after vaginal delivery was greater than after cesarean section (7.75 vs 5.34 cm^2), indicating that there might be a combined effect of pregnancy and vaginal delivery on the pelvic floor.

Regarding levator avulsion, in a recent study of van Delft *et al.* looked at the ultrasound images of the puborectalis muscle at 4 days and 3 months after first delivery.[26] This study noticed the occurrence of hematomas and abruptions of the muscle from its attachment at the pubic bone (levator ani muscle avulsion).[26] The incidence of levator ani muscle avulsion in primiparous women ranges between 12-36%.[8,27-30] In this study we found an incidence of 40% avulsions. With respect to complete bilateral avulsion we detected differences in recovery between the 3 patients. In one woman the hiatal area was consistently enlarged at Valsalva, in another it was not. These numbers are too small to draw conclusions, but studying the natural functional recovery of complete bilateral avulsions in a larger group seems to be interesting.

In conclusion, an increase in area of the levator hiatus on Valsalva maneuver and an increase in distensibility of the puborectalis muscle persist after first vaginal delivery. This may play a role in the development of pelvic floor disorders later in life. In rest and on PFMC, recovery of the hiatal area size occurs within the first 3 weeks postpartum and contractility is recovered in 6 weeks after delivery. Abnormalities in this process, mapped in frequent monitoring from 1 day until 24 weeks' after delivery, could help us in selecting women who might benefit from early intervention to prevent symptoms later in life.

References

1. MacLennan AH, Taylor AW, Wilson DH, Wilson D. The prevalence of pelvic floor disorders and their relationship to gender, age, parity and mode of delivery. *BJOG* 2000; 107 : 1460-1470.
2. Dannecker C, Anthuber C. The effects of childbirth on the pelvic-floor. *J Perinat Med* 2000; 28 : 175-184.
3. Gyhagen M, Milsom I. Prevalence of urinary incontinence (UI) 20 years after childbirth in a national cohort study in singleton primiparae after vaginal or caesarean delivery--authors' reply. *BJOG* 2013; 120 : 1152-1153.
4. Lien KC, Mooney B, DeLancey JO, Ashton-Miller JA. Levator ani muscle stretch induced by simulated vaginal birth. *Obstet Gynecol* 2004; 103 : 31-40.
5. Chan SS, Cheung RY, Yiu KW, Lee LL, Leung TY, Chung TK. Pelvic floor biometry during a first singleton pregnancy and the relationship with symptoms of pelvic floor disorders: a prospective observational study. *BJOG* 2014; 121 : 121-129.
6. Van Veelen GA, Schweitzer KJ, van der Vaart CH. Ultrasound imaging of the pelvic floor: changes in anatomy during and after first pregnancy. *Ultrasound Obstet Gynecol* 2014; 44 : 476-480.
7. Falkert A, Willmann A, Endress E, Meint P, Seelbach-Gobel B. Three-dimensional ultrasound of pelvic floor: is there a correlation with delivery mode and persisting pelvic floor disorders 18-24 months after first delivery? *Ultrasound Obstet Gynecol* 2013; 41 : 204-209.
8. Dietz HP, Lanzarone V. Levator trauma after vaginal delivery. *Obstet Gynecol* 2005; 106 : 707-712.
9. Abdool Z, Dietz HP, Lindeque BG. Prolapse symptoms are associated with abnormal functional anatomy of the pelvic floor. *Int Urogynecol J* 2017; .
10. Dietz HP, Shek C, De Leon J, Steensma AB. Ballooning of the levator hiatus. *Ultrasound Obstet Gynecol* 2008; 31 : 676-680.
11. Callewaert G, Albersen M, Janssen K, Damaser MS, Van Mieghem T, van der Vaart CH, Deprest J. The impact of vaginal delivery on pelvic floor function - delivery as a time point for secondary prevention. *BJOG* 2016; 123 : 678-681.
12. Callewaert G, Da Cunha MMCM, Sindhwani N, Sampaolesi M, Albersen M, Deprest J. Cell-based secondary prevention of childbirth-induced pelvic floor trauma. *Nat Rev Urol* 2017; .
13. Deng K, Lin DL, Hanzlicek B, Balog B, Penn MS, Kiedrowski MJ, Hu Z, Ye Z, Zhu H, Damaser MS. Mesenchymal stem cells and their secretome partially restore nerve and urethral function in a dual muscle and nerve injury stress urinary incontinence model. *Am J Physiol Renal Physiol* 2015; 308 : F92-F100.
14. Dietz HP, Shek C, Clarke B. Biometry of the pubovisceral muscle and levator hiatus by three-dimensional pelvic floor ultrasound. *Ultrasound Obstet Gynecol* 2005; 25 : 580-585.
15. Chan SS, Cheung RY, Yiu KW, Lee LL, Chung TK. Pelvic floor biometry in Chinese primiparous women 1 year after delivery: a prospective observational study. *Ultrasound Obstet Gynecol* 2014; 43 : 466-474.
16. Dietz HP, Bernardo MJ, Kirby A, Shek KL. Minimal criteria for the diagnosis of avulsion of the puborectalis muscle by tomographic ultrasound. *Int Urogynecol J* 2011; 22 : 699-704.
17. Guzman Rojas R, Wong V, Shek KL, Dietz HP. Impact of levator trauma on pelvic floor muscle function. *Int Urogynecol J* 2014; 25 : 375-380.
18. Staer-Jensen J, Siafarikas F, Hilde G, Benth JS, Bo K, Engh ME. Postpartum recovery of levator hiatus and bladder neck mobility in relation to pregnancy. *Obstet Gynecol* 2015; 125 : 531-539.
19. Tunn R, DeLancey JO, Howard D, Thorp JM, Ashton-Miller JA, Quint LE. MR imaging of levator ani muscle recovery following vaginal delivery. *Int Urogynecol J Pelvic Floor Dysfunct* 1999; 10 : 300-307.

20. Lovering RM, De Deyne PG. Contractile function, sarcolemma integrity, and the loss of dystrophin after skeletal muscle eccentric contraction-induced injury. *Am J Physiol Cell Physiol* 2004; 286 : C230-8.
21. Lovering RM, Roche JA, Bloch RJ, De Deyne PG. Recovery of function in skeletal muscle following 2 different contraction-induced injuries. *Arch Phys Med Rehabil* 2007; 88 : 617-625.
22. Hickey JT, Timmins RG, Maniar N, Williams MD, Opar DA. Criteria for Progressing Rehabilitation and Determining Return-to-Play Clearance Following Hamstring Strain Injury: A Systematic Review. *Sports Med* 2016; .
23. Gillies AR, Lieber RL. Structure and function of the skeletal muscle extracellular matrix. *Muscle Nerve* 2011; 44 : 318-331.
24. Purslow PP. Muscle fascia and force transmission. *J Bodyw Mov Ther* 2010; 14 : 411-417.
25. Alperin M, Lawley DM, Esparza MC, Lieber RL. Pregnancy-induced adaptations in the intrinsic structure of rat pelvic floor muscles. *Am J Obstet Gynecol* 2015; 213 : 191.e1-191.e7.
26. Van Delft K, Thakar R, Shobeiri SA, Sultan AH. Levator hematoma at the attachment zone as an early marker for levator ani muscle avulsion. *Ultrasound Obstet Gynecol* 2014; 43 : 210-217.
27. Dietz HP. Pelvic floor trauma in childbirth. *Aust N Z J Obstet Gynaecol* 2013; 53 : 220-230.
28. DeLancey JO, Kearney R, Chou Q, Speights S, Binno S. The appearance of levator ani muscle abnormalities in magnetic resonance images after vaginal delivery. *Obstet Gynecol* 2003; 101 : 46-53.
29. Shek KL, Dietz HP. Can levator avulsion be predicted antenatally? *Am J Obstet Gynecol* 2010; 202 : 586.e1-586.e6.
30. Shek KL, Dietz HP. Intrapartum risk factors for levator trauma. *BJOG* 2010; 117 : 1485-1492.

Appendix

	12 weeks' gestation (median (range))	1 day postpartum (median (range))	P*	1 week postpartum (median (range))	P*	2 weeks postpartum (median (range))	P*	3 weeks postpartum (median (range))
Rest	(n=20)	(n=17)		(n=19)		(n=20)		(n=19)
AP diameter (cm)	4.51(3.88 – 5.61)	6.02(4.89 – 7.02)	**0.001**	5.15(4.32 – 5.99)	**0.000**	4.98(4.23 – 6.12)	**0.001**	4.76(4.02 – 5.60)
Transverse diameter (cm)	3.51(3.04 – 4.61)	3.99(3.00 – 5.25)	**0.002**	3.61(2.52 – 4.94)	0.355	3.85(3.09 – 5.04)	**0.009**	3.67(2.90 – 4.73)
Area (cm^2)	10.67(8.23 – 14.66)	16.09(11.32 – 21.56)	**0.001**	12.71(9.09 – 15.71)	**0.027**	12.19(9.92 – 18.82)	**0.002**	10.28(8.35 – 15.97)
Contraction	(n=20)	(n=17)		(n=19)		(n=20)		(n=19)
AP diameter (cm)	3.81(3.37 – 4.83)	5.66(4.32 – 6.95)	**0.000**	4.81(4.02 – 5.58)	**0.000**	4.31(3.91 – 5.58)	**0.000**	4.45(3.69 – 5.43)
Transverse diameter (cm)	3.41(2.45 – 4.82)	3.63(2.92 – 5.36)	**0.002**	3.70(2.93 – 4.37)	**0.030**	3.78(3.18 – 4.72)	**0.002**	3.72(3.12 – 4.57)
Area (cm^2)	9.23(6.74 – 14.81)	14.14(10.64 – 22.76)	**0.000**	12.13(9.06 – 14.20)	**0.001**	11.55(9.90 – 15.82)	**0.000**	10.20(8.18 – 15.06)
Valsalva	(n=19)	(n=16)		(n=18)		(n=19)		(n=18)
AP diameter (cm)	4.46(3.58 – 6.61)	6.25(5.21 – 6.95)	**0.002**	5.45(4.89 – 6.48)	**0.002**	5.58(4.91 – 7.11)	**0.001**	5.48(4.67 – 7.00)
Transverse diameter (cm)	3.86(3.36 – 4.52)	4.16(3.60 – 6.46)	**0.011**	4.03(3.13 – 5.01)	0.098	4.43(3.45 – 5.32)	**0.000**	4.44(3.46 – 5.65)
Area (cm^2)	11.57(9.2 – 19.12)	17.88(12.18 – 22.31)	**0.004**	15.10(10.31 – 22.68)	**0.025**	17.05(12.99 – 25.29)	**0.000**	16.96(11.67 – 27.11)

Hiatal dimensions at 12 weeks' gestation and 1 day, 1, 2, 3, 4, 5, 12, 18 and 24 weeks postpartum

* compared with the same patients at 12 weeks' gestation

AP = anteroposterior (sagittal)

	12 weeks' gestation (median(range))	1 day postpartum (median(range))	P*	1 week postpartum (median(range))	P*	2 weeks postpartum (median(range))	P*	3 weeks postpartum (median(range))
Rest – contraction	(n=20)	(n=17)		(n=19)		(n=20)		(n=19)
Δ AP diameter (cm)	0.51(0.22 – 1.49)	0.24(-0.10 – 1.54)	0.102	0.43(-0.29 – 0.89)	**0.009**	0.40(-0.05 – 1.08)	**0.095**	0.33(0.06 – 0.75)
Δ Transverse diameter (cm)	0.08(-0.44 – 0.83)	0.08(-0.35 – 0.38)	0.619	0.08(-0.41 – 0.58)	0.387	0.04(-0.35 – 0.60)	0.478	-0.07(-0.45 – 0.70)
Δ Area (cm^2)	1.59(-0.15 – 3.64)	0.37(-1.20 – 3.65)	0.124	0.43(-0.87 – 2.99)	**0.018**	1.01(-0.44 – 3.00)	0.079	0.47(-1.76 – 1.96)
Valsalva – rest	(n=19)	(n=16)		(n=18)		(n=19)		(n=18)
Δ AP diameter (cm)	0.18(-0.68 – 1.45)	0.16(-0.37 – 0.72)	0.856	0.39(-0.04 – 1.49)	0.151	0.57(-0.28 – 1.54)	**0.005**	0.75(-0.18 – 2.28)
Δ Transverse diameter (cm)	0.21(-0.28 – 1.02)	0.09(-0.39 – 1.69)	0.776	0.34(-0.25 – 1.49)	0.381	0.39(0.11 – 1.21)	**0.016**	0.40(-0.17 – 1.95)
Δ Area (cm^2)	0.90(-0.99 – 8.13)	1.10(-0.43 – 3.96)	0.756	2.85(-0.30 – 9.78)	0.071	4.00(-1.77 – 12.53)	**0.006**	5.04(-0.97 – 17.03)

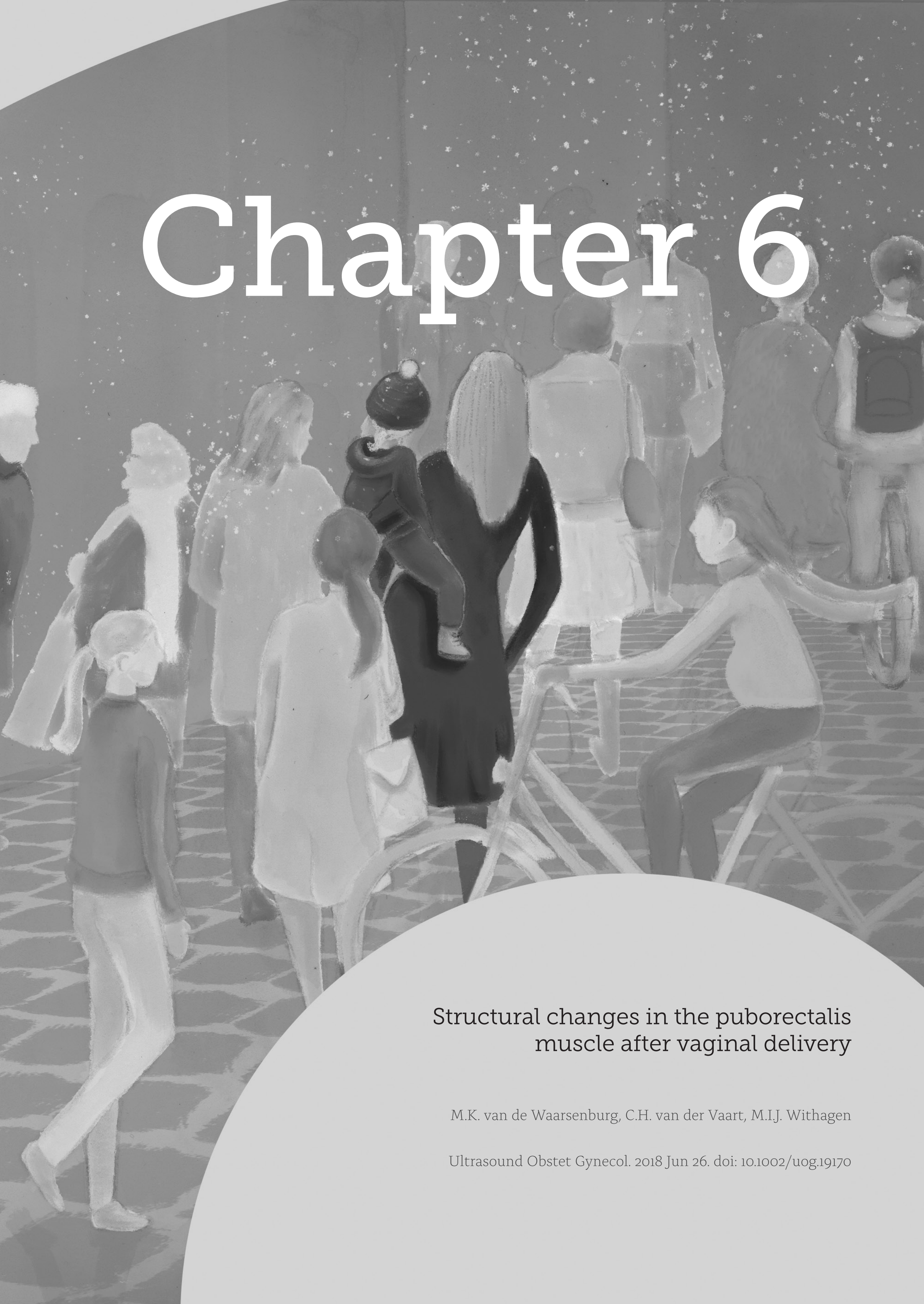
Contractility and distensibility at 12 weeks' gestation and 1 day, 1, 2, 3, 4, 5, 12, 18 and 24 weeks postpartum

* compared with the same patients at 12 weeks' gestation

AP = anteroposterior (sagittal)

	P*	4 weeks postpartum (median (range))	P*	6 weeks postpartum (median (range))	P*	12 weeks postpartum (median (range))	P*	18 weeks postpartum (median (range))	P*	24 weeks postpartum (median (range))	P*
		(n=18)		(n=20)		(n=20)		(n=19)		(n=18)	
	0.044	4.64(3.91 – 5.69)	0.117	4.75(3.99 – 6.32)	**0.026**	4.56(3.80 – 5.93)	**0.044**	4.75(3.72 – 6.20)	**0.036**	4.54(3.88 – 5.58)	0.384
	0.573	3.68(2.90 – 4.79)	0.098	3.71(3.00 – 4.61)	0.157	3.85(3.06 – 4.59)	0.104	3.83(3.29 – 5.03)	**0.009**	3.94(2.85 – 4.78)	0.286
	0.904	10.83(7.53 – 15.14)	0.446	11.08(9.16 – 18.59)	0.575	11.74(8.53 – 15.59)	0.263	11.59(8.54 – 14.53)	**0.030**	11.11(8.94 – 14.70)	0.811
		(n=18)		(n=20)		(n=20)		(n=19)		(n=18)	
	0.003	4.23(3.54 – 5.64)	**0.026**	4.35(3.52 – 5.55)	**0.005**	3.95(3.32 – 5.67)	0.117	4.04(3.49 – 5.60)	0.220	4.02(3.24 – 5.14)	0.711
	0.039	3.65(2.58 – 4.81)	0.102	3.68(2.67 – 4.68)	0.263	3.76(2.70 – 4.49)	0.089	3.90(2.68 – 4.77)	**0.001**	3.73(2.78 – 5.38)	0.058
	0.061	10.47(6.42 – 13.57)	0.085	10.88(7.36 – 16.40)	**0.016**	9.88(7.49 – 13.07)	0.313	10.51(7.92 – 14.05)	0.159	10.54(7.97 – 13.22)	0.316
		(n=17)		(n=19)		(n=19)		(n=19)		(n=17)	
	0.004	5.36(4.55 – 6.83)	**0.006**	5.44(4.08 – 6.83)	**0.001**	5.32(4.60 – 6.87)	**0.002**	5.13(4.70 – 7.20)	**0.002**	5.04(3.84 – 6.46)	0.093
	0.002	4.29(3.43 – 5.18)	**0.005**	4.36(3.48 – 5.35)	**0.001**	4.51(3.05 – 6.02)	**0.005**	4.28(3.37 – 5.64)	**0.003**	4.05(2.94 – 5.39)	0.149
	0.002	15.85(11.62 – 24.93)	**0.009**	15.42(10.35 – 25.26)	**0.001**	16.06(11.01 – 27.75)	**0.003**	17.51(12.12 – 29.55)	**0.001**	14.89(9.93 – 22.07)	0.076

	P*	4 weeks postpartum (median(range))	P*	6 weeks postpartum (median(range))	P*	12 weeks postpartum (median(range))	P*	18 weeks postpartum (median(range))	P*	24 weeks postpartum (median(range))	P*
		(n=18)		(n=20)		(n=20)		(n=19)		(n=18)	
	0.021	0.46(0.00 – 0.89)	0.085	0.59(-0.56 – 1.18)	0.702	0.48(-0.13 – 1.49)	0.737	0.60(0.12 – 1.38)	0.763	0.60(-0.77 – 1.40)	0.486
	0.212	0.06(-0.37 – 0.86)	0.459	0.01(-0.46 – 0.66)	0.360	0.08(-1.07 – 0.75)	0.985	0.10(-1.18 – 0.86)	0.809	0.04(-0.81 – 1.19)	0.074
	0.015	0.35(-2.12 – 2.81)	**0.031**	0.56(-1.89 – 5.51)	**0.037**	1.71(-1.83 – 3.50)	0.695	1.26(-2.72 – 3.67)	0.872	0.90(-2.82 – 3.07)	0.296
		(n=17)		(n=19)		(n=19)		(n=19)		(n=17)	
	0.028	0.70(-0.24 – 1.42)	0.052	0.68 (-0.34 – 1.57)	**0.017**	0.75(-0.34 – 1.41)	**0.018**	0.71(-0.19 – 1.28)	**0.008**	0.22(-0.81 – 1.56)	0.179
	0.039	0.53(0.07 – 0.92)	**0.026**	0.43(-0.02 – 1.52)	**0.007**	0.58(-0.32 – 1.86)	0.056	0.26(-0.14 – 1.50)	0.136	0.35(-0.19 – 1.25)	0.256
	0.010	4.12(-0.94 – 11.02)	**0.001**	5.03(1.17 – 14.84)	**0.002**	4.18(0.24 – 14.91)	**0.002**	3.79(-0.23 – 15.39)	**0.001**	2.75(0.05 – 10.22)	**0.028**



Chapter 6

Structural changes in the puborectalis muscle after vaginal delivery

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Abstract

Objective: This study is designed to evaluate the structural composition of the puborectalis muscle at several moments in time, by the use of echogenicity and area measurements, in order to explore recovery after first vaginal delivery.

Methods: Twenty nulliparous women with a singleton pregnancy received 3D/4D transperineal ultrasound assessments at rest, contraction and Valsalva at 12 weeks' gestation and at 1 day and 1, 2, 3, 4, 6, 12, 18 and 24 weeks after vaginal delivery. The puborectalis muscle was delineated for measurements of the mean echogenicity of the puborectalis muscle (MEP) and area (PMA). To assess changes in MEP and PMA over time, we used a linear mixed model analysis. The exact days after delivery during the ultrasound investigations were used as a covariate.

Results: After delivery the MEP is statistical significantly decreased compared to the MEP during pregnancy at all time points. The MEP values increased significantly over time from day 1 to 24 weeks after delivery. Although not statistically significant we observed a remarkable drop in MEP between 3 and 4 weeks in all maneuvers. The PMA remained constant after delivery at rest and during Valsalva maneuver.

Conclusion: As compared to pregnancy levels we observed a sharp decrease in echogenicity soon after delivery, which is most likely caused by stretch trauma of the puborectalis muscle and subsequent formation of (micro) hematoma and edema. Afterwards the increasing MEP may reflect the disappearance of hematoma and edema and also the formation of connective tissue and scar tissue.

Introduction

Vaginal delivery causes damage to the pelvic floor, partly due to stretching of the levator ani muscle, which stretches up to 2.5 times its original length.[1] This damage could lead to the development of pelvic floor disorders such as pelvic organ prolapse and urinary incontinence, often with a time latency between trauma and symptoms.[2] The extent of the trauma and differences in trauma healing after delivery may be associated with the development of these pelvic floor disorders.

Little is known about normal recovery of the puborectalis muscle after vaginal delivery. With repeated 3D perineal ultrasound measurements of the hiatal dimensions between 1 day and 24 weeks after first vaginal delivery, we were able to show that an important part of the functional recovery occurs within the first three weeks after delivery. The hiatal area, in rest and contraction, returned to values comparable to early pregnancy in the time interval of 3 weeks after delivery.[3] However, stretch of the muscle, reflected by distensibility of the hiatal area during Valsalva, remained consistently increased up until 24 weeks.[3] This implicates that there are persistent structural changes in the puborectalis muscle after delivery.

Echogenicity measurements on the ultrasound images can be used to assess the structure of muscle tissue. Echogenicity reflects the proportion of muscle cells and extracellular matrix in a muscle with values between 0 and 255. Muscle cells appear dark on the ultrasound image (low echogenicity) and extracellular matrix, such as fat or collagen, appears bright on the ultrasound image (high echogenicity). These echogenicity measurements have proven to be of clinical use in neuromuscular disorders and orthopaedics.[4,5]

Knowledge about the structural composition of the puborectalis muscle during recovery after delivery may help us understanding why functional recovery of the distensibility does not occur. In addition, for future use and results of regenerative techniques that aim at improving structural recovery of the puborectalis muscle after delivery, we need surrogate markers for possible damage, since symptoms often appear years after delivery.[6,7]

This study is designed to evaluate the structural composition of the puborectalis muscle at several moments after first vaginal delivery, by the use of echogenicity and area measurements.

Methods

This study is part of a prospective multicenter cohort study on the association between the mean echogenicity of the puborectalis muscle (MEP) and mode of delivery. The first 20 consecutive patients of the university medical center Utrecht, who delivered vaginally and gave informed consent for an extensive follow up study postpartum during 24 weeks, were selected. These 20 women were part of 306 nulliparous women with a singleton pregnancy and good knowledge of the Dutch language that were included between March 2015 and January 2017. Exclusion criteria were a history of pelvic organ prolapse surgery, incontinence surgery, surgery in the uterus by which they had to deliver by Cesarean section and connective tissue disease or an inability to perform a maximum Valsalva maneuver because of cardiac or pulmonary disease. All women gave informed consent and the study was approved by the Institutional Human Research Ethics Committee (reference 14/482).

3D/4D transperineal ultrasound assessments were performed at 12 weeks' gestation and at 1 day and 1, 2, 3, 4, 6, 12, 18 and 24 weeks after vaginal delivery. The images after delivery were made at our clinic or at the home of the women, according to her preference. The ultrasound devices used were either the GE Voluson 730 Expert system (GE Healthcare, Zipf, Austria) or the portable Voluson *i* (GE Healthcare, Zipf, Austria). During the examination, perineal volume images were made at rest, contraction and on Valsalva maneuver, with the participant in supine position and with an empty bladder. Each examination was recorded three times and the obtained volume imaging datasets were stored on a hard disk.

Offline analysis was performed using 4D View 7.0 (GE Medical Systems Kretztechnik, Zipf, Austria) and Matlab® R2010a (MathWorks, Natick, MA) software. The plane of minimal hiatal dimension of the levator hiatus in axial position was selected in 4D View and transported to Matlab to delineate the puborectalis muscle for measurements of the MEP and area of the puborectalis muscle (PMA).[8,9]

Despite the same intensity value settings of both Voluson ultrasound devices, the echogenicity was not comparable without using a conversion factor, as already determined by Pillen *et al.*[4] Therefore, we developed and validated a conversion factor, using an ATS type 570 ultrasound phantom (ATS Laboratories, Bridgeport, USA), according to the method of Pillen *et al.*[4] The look up table of each ultrasound device was corrected and the echogenicity values per density of the portable Voluson *i* were corrected to the "gold standard" Voluson 730.

To define the presence of a levator ani muscle avulsion the levator urethra gap was measured as previously described by Dietz *et al*.[10,11] To measure the MEP and PMA in women with a levator ani muscle avulsion, care was taken to clearly outline the area of the muscle itself when it was detached from the pubic bone.

Statistical analysis was performed using SPSS version 22.0 for Windows. To assess changes in MEP and PMA over time, we used a linear mixed model analysis. The exact days after delivery during the ultrasound investigations were used as a covariate. Scatter plots were used to visualize the statistically significant patterns. $P<0.05$ was considered statistically significant.

Results

A total of 188 volume datasets of the 200 planned perineal ultrasounds were analyzed (Flowchart, Figure 1).

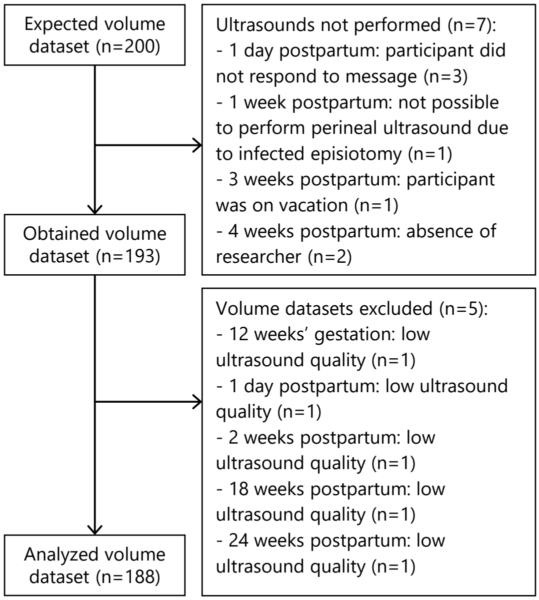


Figure 1. Diagram illustrating the number of volume datasets obtained and analyzed of the 20 participants.

The analyzed datasets per time interval contained at least 2 well recorded volumes out of the 3 maneuvers (rest, contraction, Valsalva). The median age of the women was 32 years, 95% was Caucasian and median gestational age at delivery was 40.1 weeks. One woman delivered prematurely at 31 weeks' gestation.

Mean echogenicity of the puborectalis muscle

The mean MEP after delivery is statistical significantly decreased at all time intervals compared to the MEP during pregnancy (MEP rest 143, MEP contraction 140, MEP Valsalva 141) (p-values between 0.000 and 0.002). The mean MEP values at 24 weeks after delivery (MEP rest 117, MEP contraction 108, MEP Valsalva 111) were significantly increased compared to day 1 after delivery (MEP rest 83, MEP contraction 87, MEP Valsalva 90) (rest p=0.000, contraction p=0.009, Valsalva p=0.002) (Figure 2).

The mixed model analysis of the MEP after delivery showed a statistically significant increase over time in rest (an increase of 0.16/day with a p-value <0.001) and during contraction (an increase of 0.11/day with a p-value of 0.008), during Valsalva the increase was 0.06/day with a p-value of 0.164. Although not statistical significant there appeared to be a small decrease in MEP values in all maneuvers between 3 and 4 weeks.

Area of the puborectalis muscle

The mean PMA remained constant after delivery at rest and during Valsalva maneuver. During contraction a significantly increased mean PMA 1 day after delivery compared to pregnancy (9.05 cm^2 versus 7.68 cm^2, p=0.017) and a significantly decreased mean PMA at 24 weeks after delivery (6.68 cm^2 versus 7.55 cm^2, p=0.020) compared to pregnancy was found. The mean PMA at contraction decreased significantly in the postpartum period (1 day after delivery versus 24 weeks after delivery (p=0.005)) (Figure 3).

The mixed model analysis of the area after delivery showed a statistically significant decrease over time during contraction (a decrease of 0.008/day with a p-value of 0.003, figure), in rest an increase of 0.000064/day with a p-value of 0.979 and during Valsalva a decrease of 0.003/day with a p-value of 0.359.

Levator ani muscle avulsions

Complete levator ani muscle avulsions, 3 bilateral and 2 right, were seen in 5/20 (25%) women in our study. The 3 women with bilateral complete levator ani muscle avulsions showed an equal shape of the curves of median MEP and PMA as in the 17 remainder women (Figure 4 and 5). However, the PMA was smaller in the women with a complete bilateral avulsion.

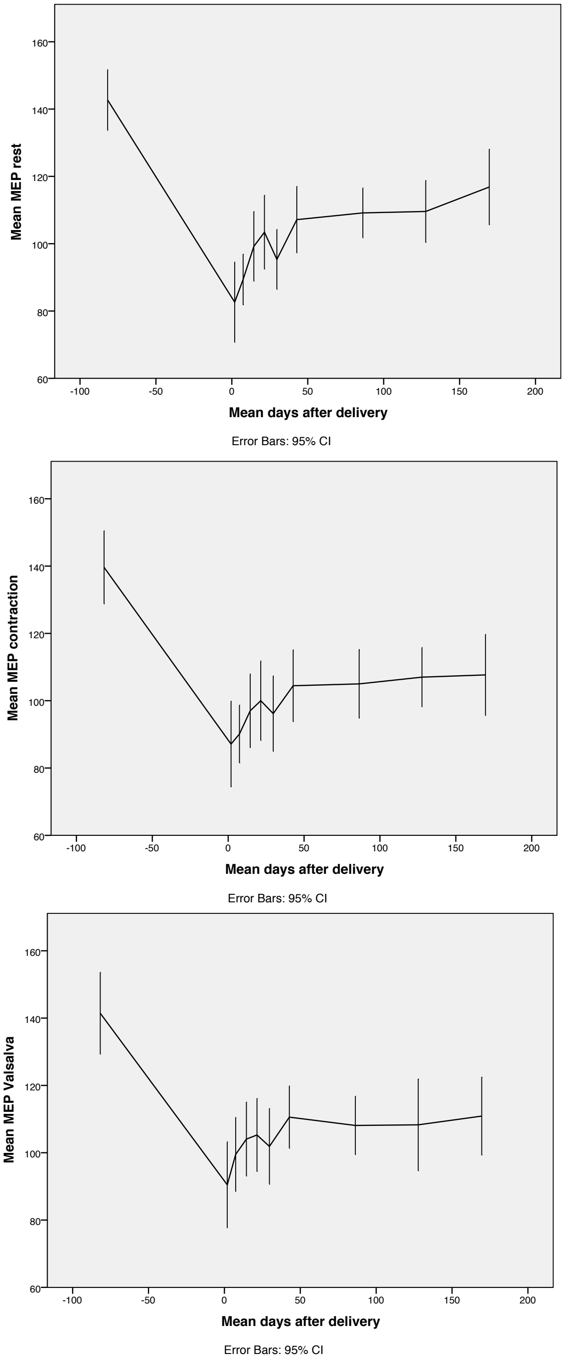


Figure 2. Mean MEP at rest, contraction and on Valsalva maneuver at 12 weeks' gestation and at 1 day and 1, 2, 3, 4, 6, 12, 18 and 24 weeks after vaginal delivery with 95%-confidence intervals.

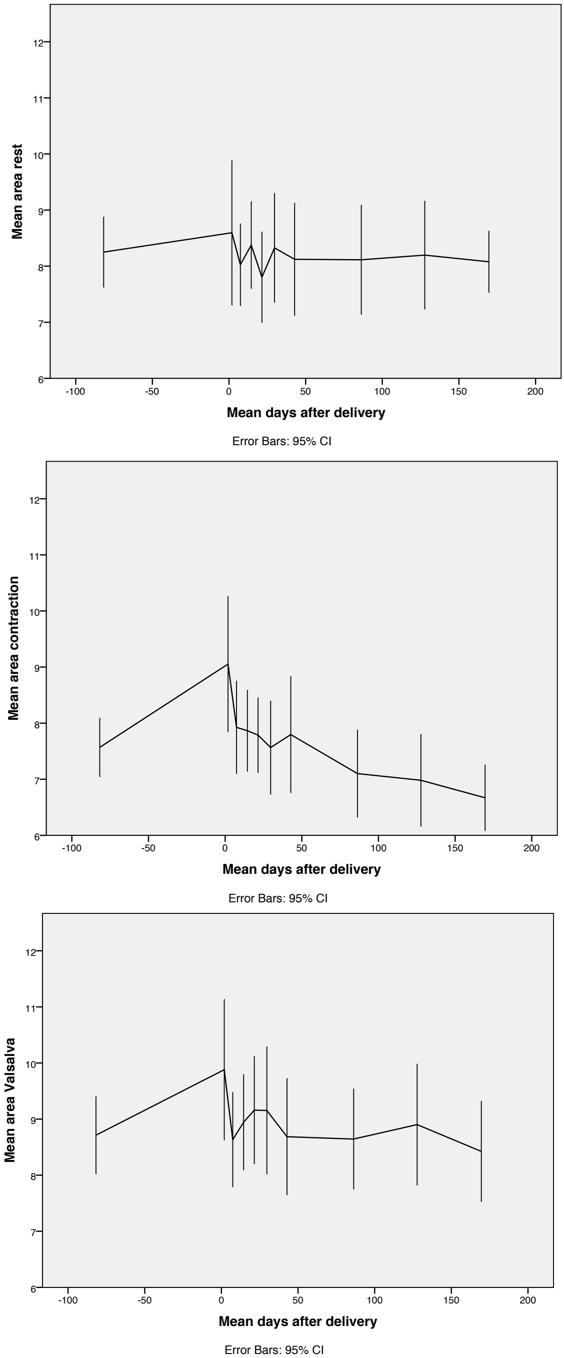


Figure 3. Mean PMA at rest, contraction and on Valsalva maneuver at 12 weeks' gestation and at 1 day and 1, 2, 3, 4, 6, 12, 18 and 24 weeks after vaginal delivery with 95%-confidence intervals.

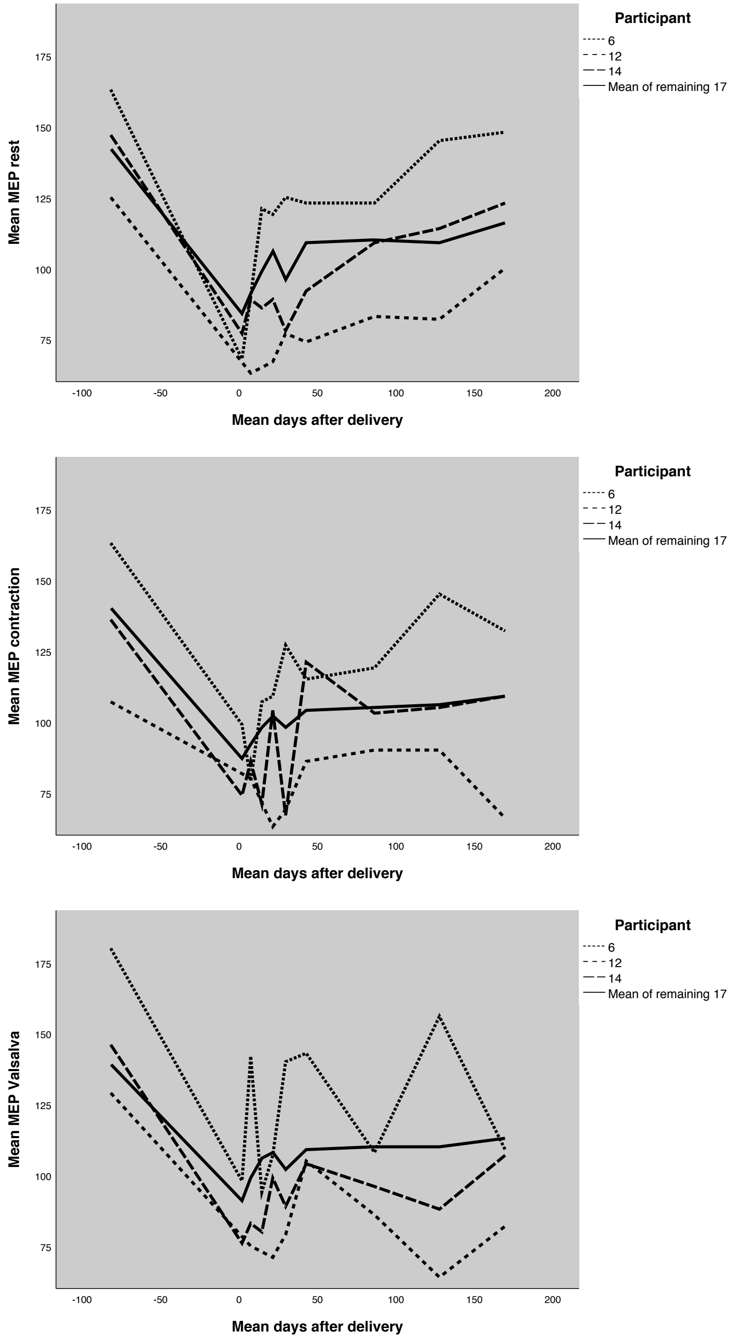


Figure 4. Mean MEP at rest, contraction and on Valsalva maneuver at 12 weeks' gestation and at 1 day and 1, 2, 3, 4, 6, 12, 18 and 24 weeks after vaginal delivery in the 3 women with a complete bilateral avulsion and the median of the 17 remaining women.

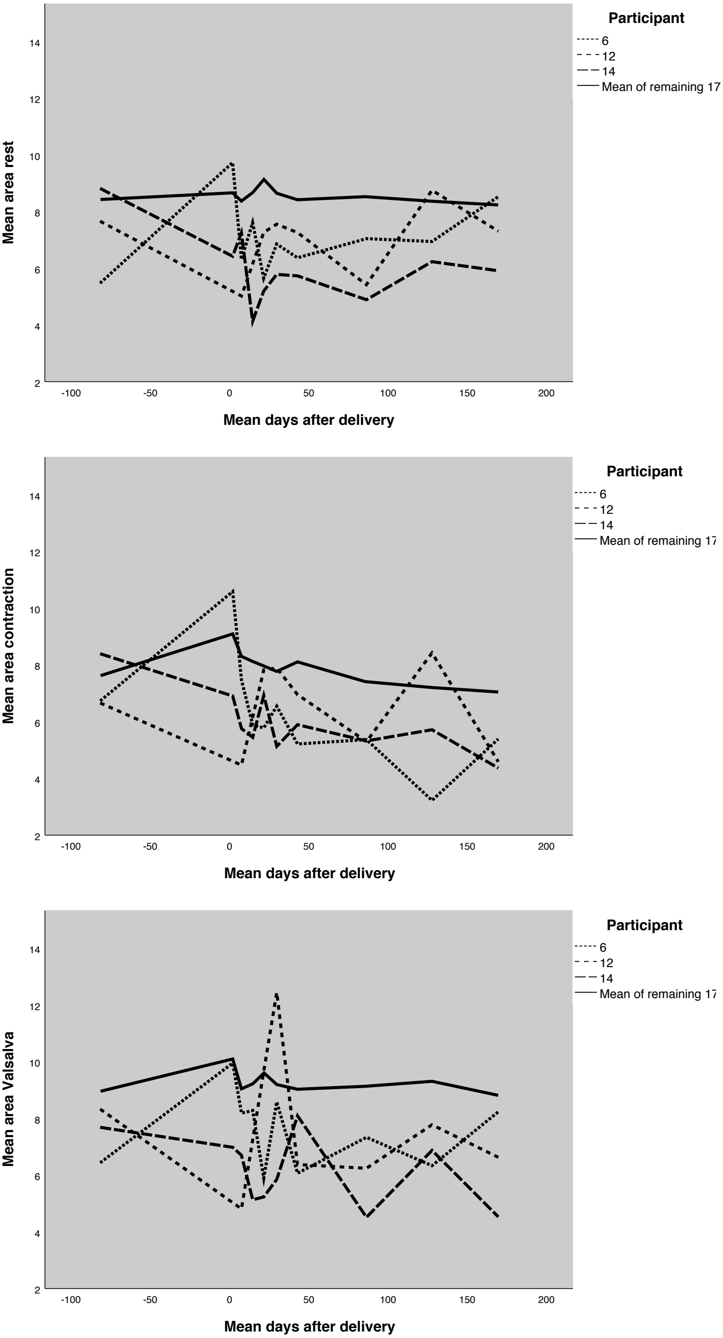


Figure 5. Mean PMA at rest, contraction and on Valsalva maneuver at 12 weeks' gestation and at 1 day and 1, 2, 3, 4, 6, 12, 18 and 24 weeks after vaginal delivery in the 3 women with a complete bilateral avulsion and the median of the 17 remaining women.

Discussion

Echogenicity of the puborectalis muscle after delivery, is significantly decreased compared to echogenicity during pregnancy, with the lowest echogenicity 1 day after delivery and slowly increasing echogenicity up to 24 weeks after delivery, not reaching pregnancy values.

During contraction a significantly increased PMA of the puborectalis muscle was seen at 1 day after delivery compared to pregnancy values and a significantly decreased PMA at 24 weeks after delivery compared to pregnancy values.

Three complete bilateral levator ani muscle avulsions were seen in this pilot study, with an equal shape of the MEP and PMA curves compared to the women without a complete bilateral levator ani muscle avulsion, however the PMA was smaller.

The major limitation of this study is the small study population. We decided to do this pilot study with 20 participants because there were no available data from literature to do a sample size calculation and because of the large number of ultrasound follow up moments and time investment for the women. The absence of pre-pregnancy values of MEP and PMA is another limitation; possible changes that occurred between the non-pregnant and pregnant state may have provided extra information for proper interpretation of the postpartum values. Furthermore, it would be ideal to use histology of the muscle for the interpretation of the MEP, obviously this is not possible in our population. The fourth limitation is that the population consisted of mainly Caucasian women with a median age of 32, so the conclusion may not be applicable to other groups. The final limitation is that one woman delivered prematurely (at 31 weeks' gestation), however as values of MEP and PMA were comparable to the group median and a partial levator ani muscle avulsion was seen, we decided not to exclude this woman.

The major strength of the study is the longitudinal study design with frequent follow up moments shortly after delivery up until 24 weeks postpartum. Another strength in the study design is the possibility for the participants to have the ultrasounds made at their home, this limited the loss to follow up.

Mean echogenicity and area of the puborectalis muscle before delivery

In the study of Grob *et al.*, the changes in the MEP during pregnancy (at 12 and 36 weeks) and 6 months after delivery were measured.[12] The MEP values during pregnancy (at both moments in time) were significantly higher compared to the MEP values 6 months after delivery.[12] Data in this study confirm these findings, all postpartum echogenicity values are significantly lower compared to values during pregnancy. As the values of the MEP at 36 weeks' gestation in the study of Grob *et al.* are as high or even higher than at 12 weeks' gestation, we assume that the values just before delivery are comparable.[12]

Mean echogenicity and area of the puborectalis muscle after delivery

One day after delivery we see a sharp decrease in MEP, most likely due to (micro)hematoma and edema, since fluid appears dark on ultrasound image.[13-15] The (small) increase in PMA at 1 day after delivery (statistically significant during contraction) is probably also due to edema and (micro)hematoma.

From one day on, we show a slow increase in echogenicity postpartum. This increasing MEP may reflect the recovery process after stretch trauma in muscles. The general repair process of skeletal muscles contains three phases; in phase 1, within the first day after injury,[16] tearing and necrosis of myofibrils, the formation of hematoma and inflammation is seen.[17-19] Phase 2, around 10 days after a traumatic event,[20] consists of repair, remodeling, regeneration of myofibrils and production of connective scar tissue; and phase 3, which starts three weeks after injury, includes maturation of regenerated myofibrils, contraction, reorganization of the scar tissue and recovery of function.[17,18] The short drop in MEP values in all maneuvers between 3 and 4 weeks could be a result of the end of the rapid proliferative phase, in which collagen is formed (high echogenicity), and the start of the consolidation phase. Over time, in the interval of 24 weeks after delivery, the echogenicity increases and stabilizes, most likely due to the disappearance of hematoma and edema and the formation of scar tissue that appears bright on the ultrasound image.[21] The significant decrease in PMA during contraction in the postpartum period, could also be the resultant of the disappearance of hematoma and edema and/or a better ability to contract (structural recovery, towards recovery of function). Ideally, we should have been able to link this recovery of muscular structure and function to the experience of symptoms (later in life) to explore the clinical merits of our findings.

Levator ani muscle avulsions

With respect to the data of the 3 women with a complete bilateral avulsion we only noticed a smaller PMA as compared to the other women in the study. Although statistical analysis was inappropriate due to the small sample size, this observation is interesting to test in future studies. We hypothesize that the muscle retracted due to the abruption from the bone, but since we have no volume data of the puborectalis muscle, we aren't able to confirm this.

It would be interesting to investigate the recovery pattern of women with a primary Cesarean section to compare this pattern with a vaginal delivery. We would recommend to do the investigations at least before delivery,1 day after delivery and 6 weeks after delivery.

Ideally, follow up of all recovery studies encloses the moment in time in which women may start experience symptoms so that the ultrasound examinations can be repeated, analyzed and maybe linked to different recovery patterns compared to women without symptoms.

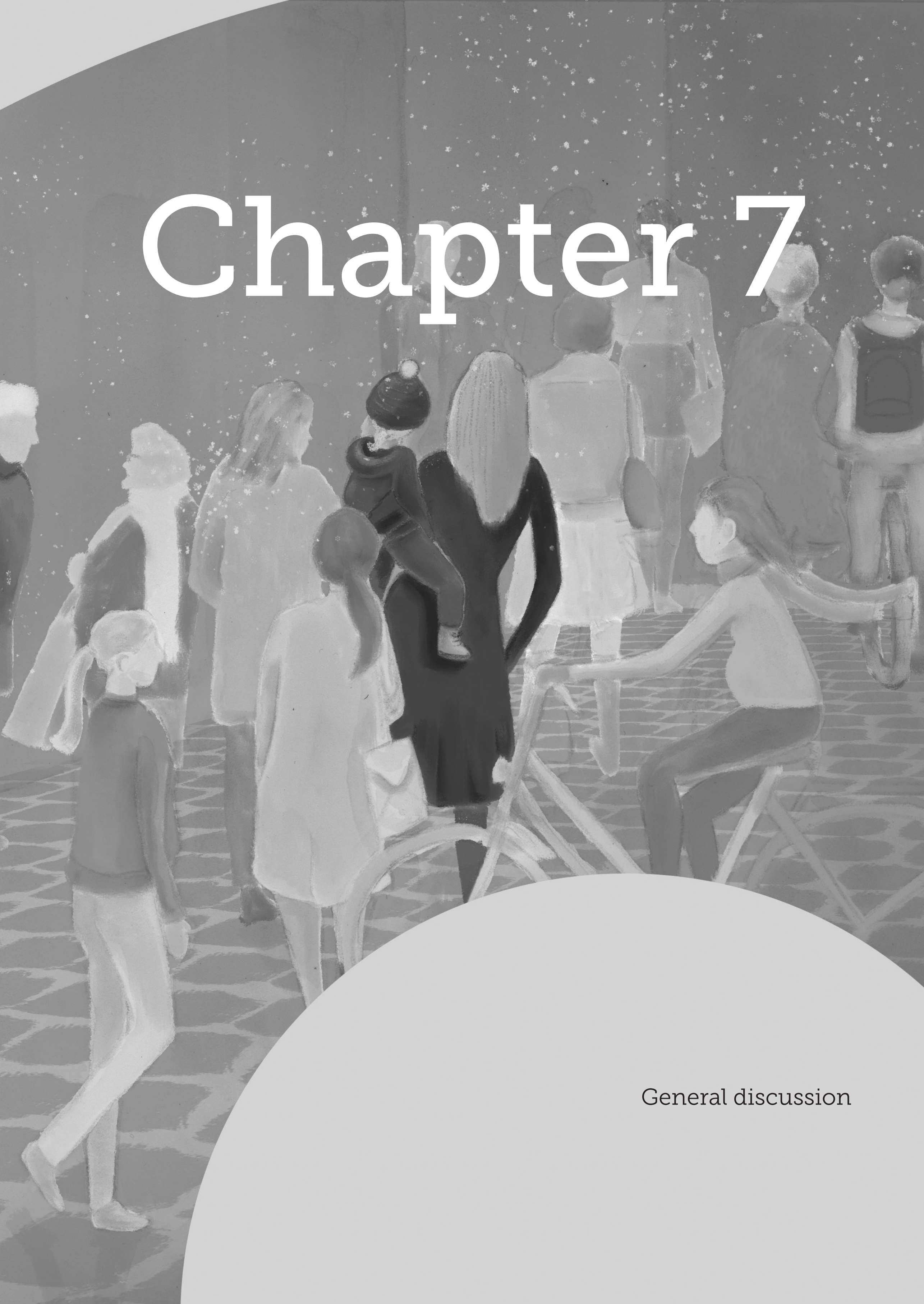
In conclusion, as compared to pregnancy levels we observed a sharp decrease in echogenicity soon after delivery, which is most likely caused by stretch trauma of the puborectalis muscle and subsequent (micro) hematoma formation and edema. Afterwards the increasing MEP may reflect the disappearance of hematoma and edema and also the formation of connective tissue.

References

1. Lien KC, Mooney B, DeLancey JO, Ashton-Miller JA. Levator ani muscle stretch induced by simulated vaginal birth. *Obstet Gynecol* 2004; 103 : 31-40.
2. Skinner EM, Dietz HP. Psychological and somatic sequelae of traumatic vaginal delivery: A literature review. *Aust N Z J Obstet Gynaecol* 2015; 55 : 309-314.
3. Van de Waarsenburg MK, Verberne EA, van der Vaart CH, Withagen MIJ. Recovery of the puborectalis muscle after vaginal delivery: an ultrasound study. *Ultrasound Obstet Gynecol* 2017; Dec 5. doi: 10.1002/uog.18976. [Epub ahead of print].
4. Pillen S, van Alfen N. Skeletal muscle ultrasound. *Neurol Res* 2011; 33 : 1016-1024.
5. Vanhoenacker FM, Baert AL, Faletti C, Maas M, Gielen JL. *Imaging of Orthopedic Sports Injuries*. Springer Berlin Heidelberg, 2007.
6. Woodley SJ, Boyle R, Cody JD, Morkved S, Hay-Smith EJC. Pelvic floor muscle training for prevention and treatment of urinary and faecal incontinence in antenatal and postnatal women. *Cochrane Database Syst Rev* 2017; 12 : CD007471.
7. Dissaranan C, Cruz MA, Kiedrowski MJ, Balog BM, Gill BC, Penn MS, Goldman HB, Damaser MS. Rat mesenchymal stem cell secretome promotes elastogenesis and facilitates recovery from simulated childbirth injury. *Cell Transplant* 2014; 23 : 1395-1406.
8. Dietz HP, Shek C, Clarke B. Biometry of the pubovisceral muscle and levator hiatus by three-dimensional pelvic floor ultrasound. *Ultrasound Obstet Gynecol* 2005; 25 : 580-585.
9. Grob AT, Veen AA, Schweitzer KJ, Withagen MI, van Veelen GA, van der Vaart CH. Measuring echogenicity and area of the puborectalis muscle: method and reliability. *Ultrasound Obstet Gynecol* 2014; 44 : 481-485.
10. Dietz HP, Bernardo MJ, Kirby A, Shek KL. Minimal criteria for the diagnosis of avulsion of the puborectalis muscle by tomographic ultrasound. *Int Urogynecol J* 2011; 22 : 699-704.
11. Guzman Rojas R, Wong V, Shek KL, Dietz HP. Impact of levator trauma on pelvic floor muscle function. *Int Urogynecol J* 2014; 25 : 375-380.
12. Grob AT, Withagen MI, van de Waarsenburg MK, Schweitzer KJ, van der Vaart CH. Changes in the mean echogenicity and area of the puborectalis muscle during pregnancy and postpartum. *Int Urogynecol J* 2016; 27 : 895-901.
13. Kullmer K, Sievers KW, Rompe JD, Nagele M, Harland U. Sonography and MRI of experimental muscle injuries. *Arch Orthop Trauma Surg* 1997; 116 : 357-361.
14. Mattiussi G, Baldassi PT, Pasta G, Burani A, Moreno C. Perivascular Adductor Longus muscle injury: Ultrasound and Magnetic Resonance Imaging findings. *Muscles Ligaments Tendons J* 2017; 7 : 376-387.
15. Peixinho CC, Ribeiro MB, Resende CM, Werneck-de-Castro JP, de Oliveira LF, Machado JC. Ultrasound biomicroscopy for biomechanical characterization of healthy and injured triceps surae of rats. *J Exp Biol* 2011; 214 : 3880-3886.
16. Cannon JG, St Pierre BA. Cytokines in exertion-induced skeletal muscle injury. *Mol Cell Biochem* 1998; 179 : 159-167.
17. Jarvinen TA, Jarvinen TL, Kaariainen M, Kalimo H, Jarvinen M. Muscle injuries: biology and treatment. *Am J Sports Med* 2005; 33 : 745-764.
18. Kaariainen M, Jarvinen T, Jarvinen M, Rantanen J, Kalimo H. Relation between myofibers and connective tissue during muscle injury repair. *Scand J Med Sci Sports* 2000; 10 : 332-337.
19. Fernandes TL, Pedrinelli A, Hernandez AJ. Muscle Injury - Physiopathology, Diagnosis, Treatment and Clinical Presentation. *Rev Bras Ortop* 2015; 46 : 247-255.

20. Kaariainen M, Kaariainen J, Jarvinen TL, Sievanen H, Kalimo H, Jarvinen M. Correlation between biomechanical and structural changes during the regeneration of skeletal muscle after laceration injury. *J Orthop Res* 1998; 16 : 197-206.
21. Dimpfl T, Jaeger C, Mueller-Felber W, Anthuber C, Hirsch A, Brandmaier R, Schuessler B. Myogenic changes of the levator ani muscle in premenopausal women: the impact of vaginal delivery and age. *Neurourol Urodyn* 1998; 17 : 197-205.

Chapter 7



General discussion

General discussion

Pregnancy and childbirth are strongly associated with pelvic floor disorders such as pelvic organ prolapse and urinary incontinence. These disorders are highly prevalent conditions with a negative impact on quality of life.[1] The mechanisms behind the association between pregnancy and childbirth and pelvic floor disorders are complex.[2] Changes in the structural composition of the pelvic floor during pregnancy and after delivery, and the normal recovery after pregnancy and vaginal delivery are largely unknown and represented the core focus of the current thesis. This chapter summarizes the interpretation, relevance and clinical implications of the main findings generated by various studies represented in this thesis, and outlines recommendations for future research.

The outline of this thesis was:

- To develop a reliable method to measure the area and mean echogenicity of the mid-urethra, as a representation of the urethral sphincter structural composition. And to assess the effect of pregnancy by measuring changes in area and echogenicity of the mid-urethra over time in pregnancy and after delivery (Chapter 2).
- To assess the association between the puborectalis muscle area and mean echogenicity and stress urinary incontinence symptoms in pregnancy and after delivery (Chapter 3).
- To confirm our previous findings that the mean echogenicity of the puborectalis muscle, area and hiatal dimension measurements during pregnancy are associated with the mode of delivery. In addition, the association between the echogenicity of the cervix and vastus lateralis muscle, the latter as a non-pelvic muscle, with the mode of delivery was investigated (Chapter 4).
- To describe normal recovery of the pelvic floor by means of repeated ultrasound measurements after first vaginal delivery. In chapter 5 we assess the changes in hiatal dimensions in rest but also in contraction and Valsalva. In chapter 6 we focus on changes in the mean echogenicity of the puborectalis muscle (MEP) as marker of structural changes.

Stress urinary incontinence in pregnancy and after delivery

The most common finding in women with stress urinary incontinence (SUI) is a hypermobility of the proximal urethra during increased intra-abdominal pressure, resulting in leakage of urine.[3] The current literature on transperineal ultrasound shows an increase in urethral mobility on Valsalva in women with SUI after delivery (more caudal and dorsal position), indicating loss of support.[4-12]

However, normal urethral function depends not only on the support of the urethra by the peri-urethral ligaments, but also on the intrinsic closure mechanism of the urethral musculature.[13] As marker for the urethral sphincter integrity, and possibly its contractile properties, we chose to measure the mid-urethral echogenicity and area, in pregnancy and after delivery. After establishing that the method we developed showed a good intra- and interobserver reliability we studied the changes during and after first pregnancy. The mid-urethral mean echogenicity of our study group with the pelvic floor at rest was significantly higher during pregnancy as compared to 6 months after delivery. The echogenicity of the urethra during a pelvic floor contraction did not differ between pregnancy and postpartum measurements. The most likely explanation for the higher echogenicity during pregnancy is related to the fact that pregnancy itself induces an increase in fat storage and collagen in muscle tissue.[14-16] Both will increase echogenicity as compared to non-pregnant values. This is in line with our observation that the area of the urethra during pregnancy is also significantly larger as compared to after delivery. More fat and collagen will increase the volume of tissue, representing itself in our case as an increased area. The finding of a different ratio between muscle cells and extra cellular matrix (ECM)/fat cells, in favor of the latter, might indicate that the resting pressure of the urethra, as function of its muscle content, during pregnancy decreases. This could be an explanation for the fact that many women develop SUI during the cause of pregnancy. The association between pregnancy, urethral function, bladder neck position and the occurrence of SUI has been investigated before. Meyer *et al.* demonstrated that the maximal urethral closure pressure (MUCP) decreases during pregnancy.[17] The change in ratio between muscle cells and ECM we observed may be a good explanation for this finding. In addition, they found that the MUCP was significantly lower in women with SUI during pregnancy as compared to those without. Because of the latter and the higher urethral echogenicity during pregnancy, we did an additional first analysis on SUI and the urethral echogenicity and area. We found no correlations between the urethral echogenicity and area between women with and without SUI during pregnancy and after delivery.[(unpublished data)] In earlier research, a smaller volume/thickness of the urethral sphincter was demonstrated in women with SUI.[18,19] A limitation of our study is the fact that we examined the area instead of volume. We have to determine however, if time consuming urethral volume measurements are clinically more relevant than single mid-urethral area measurements.[20]

A better understanding of the anatomy and function of the mid-urethra during and after pregnancy is now available by reliable measurements of the mid-urethral area (as surrogate marker for the volume) and mean echogenicity (as a resultant of structure). The clinical merits of our findings need to be determined since the mid-urethral echogenicity and area were not correlated to SUI in our sample.

The loss of peri-urethral support as part of the pelvic floor leads to hypermobility of the urethra which results in leakage of urine during increased intra-abdominal pressure.[21] The puborectalis muscle, as part of the levator ani muscle complex, is involved in closing of the genital hiatus and lifting and compression of the urethra against the pubic bone. The contractile and relaxation abilities of the puborectalis muscle can be indirectly assessed by measuring changes in hiatal dimensions with 3D ultrasound. It has been demonstrated that SUI during pregnancy is associated with an increased transverse diameter of the levator hiatus.[21] However, as stated, hiatal dimensions are an indirect measure of puborectalis muscle function. Puborectalis muscle function will be dependent on the structural integrity of the muscle itself. With the use of calculating the mean echogenicity of the puborectalis muscle (MEP), as marker of the structural composition, we were able to show that women with SUI after delivery had a statistically significant higher MEP as compared to continent women. However, the effect size of this finding was small (0.30). This indicates that the ratio between muscle cells and ECM shifted towards the ECM in women with SUI. Literature on muscle injuries shows that scar tissue formation, which will represent itself as a higher echogenicity, is associated with a loss of contractility function of the muscle.[22,23] If the increase in ECM in the puborectalis muscle indeed indicates that this will compromise its contractile function, its contribution to the urethral support may be compromised and hypermobility of the urethra occurs. Some limitations of our study have to be addressed. Estrogens play an important role in the wound healing process and a low estrogen status is associated with poorer healing.[24-26] Therefore, depending on their estrogen status, women may have been in different stages of recovery during the time of scanning. During breastfeeding, estrogen levels will be low and this may have an effect on the stage of recovery of levator ani muscle injury at time of image acquisition. Unfortunately, we did not collect information on breastfeeding or return to normal menstrual cycle. A second limitation is the fact that the effect size of our finding was low. This indicates that there will be a substantial overlap of the MEP values between the group of women with and without SUI. This will limit its practical use as potential marker of damage to the puborectalis muscle as predictor of SUI. On the other hand, the contractile function of the puborectalis muscle can be trained. If we are able to identify women with major degrees of damage, reflected as a high MEP, specialized and early training during the regeneration process may enhance functional outcome after healing. This potential predictive role of MEP measurement after delivery for the development of symptoms and need for early intervention will be subject of future studies. Finally, no differences in the MEP between women with and without SUI during pregnancy were found. We hypothesize that this lack of difference is based on the fact that injury due to delivery has not yet occurred. Apparently, the mechanism of developing SUI during pregnancy is different from that of SUI after childbirth.

Echogenicity during pregnancy and modes of delivery

Most caesarean sections (CS) in term pregnancies with the fetus in cephalic presentation are performed because of failure to progress (FTP) or for suspected fetal distress (FD).[27,28] Predictive models, with ante- and intra-partum characteristics that are related to the mode of delivery, have been developed to council women for their mode of delivery.[29-40] Since the levator ani muscle plays an important role in delivery, it may well be that abnormalities in its structure or function are associated with failure to deliver vaginally. In our previous studies we showed that women who delivered by CS due to FTP had a statistically significant smaller transverse diameter of the levator hiatus, a smaller levator hiatal area and a lower MEP during contraction at 12 weeks' pregnancy compared to women with a vaginal delivery.[41,42] However, the number of women with a CS due to FTP was limited and the difference in MEP was only observed during contraction. Our primary aim was to confirm our previous observations on MEP and hiatal dimensions in women who delivered by CS due to FTP. The effect of pregnancy on muscle tissue composition may also differ between the pelvic floor and other muscles. Since no differences in the ECM of the tibialis muscle in pregnant rats was showed, the effect of pregnancy on the composition of muscles may differ between the pelvic floor and other muscles.[15,43] Therefore we also assessed the association between the echogenicity of the cervix and the vastus lateralis muscle and mode of delivery. We were unable to confirm the previous results of a smaller MEP and hiatal transverse diameter and area during contraction at 12 weeks' pregnancy in women with a CS due to FTP. In addition, no association between the echogenicity of the cervix and vastus lateralis muscle and mode of delivery is found. Possible explanations for the lack of difference in the different modes of delivery of this study are considered. A difference in the decision-making process towards a CS, a difference in delineation of the puborectalis muscle between the studies and a difference in the instruction to do a proper maximal contraction maneuver were explored, but were not accountable explanations for the difference between our study results. We did find a mean 20 points lower MEP compared to the previous study despite the use of the same ultrasound equipment. The mean lower MEP was explainable due to the higher depth in the ultrasound images of the recent study. The depth exceeds 7.4cm, and changes in the beamforming of the ultrasound machine may have resulted in the lower echogenicity we found. However, since we observed a lower MEP in all groups we do not think this may have affected the comparison between groups.

If there is an association between the echogenicity of the cervix and a CS due to FTP, we expect a lower echogenicity in this group. A lower echogenicity reflects less ECM and less collagen. With a predominance of muscle cells, the process of ripening and dilatation of the cervix, as a resultant of weakening of the collagen matrix, is more likely to be obstructed. With failure to progress as a consequence. We were unable to confirm this hypothesis. Since the effect of pregnancy on muscle tissue composition could differ between the pelvic floor and non-pelvic floor muscles, as seen in the tibialis muscle of rats, we hypothesized that there is no association between the echogenicity of the vastus lateralis muscle and the modes of delivery.[15,44] We found no association between the echogenicity of the vastus lateralis muscle and the modes of delivery. In conclusion: In a second, independent multicenter dataset we were unable to conform our previous finding that the hiatal dimensions and MEP on contraction were statistically significant associated with the mode of delivery. Additionally, no difference in echogenicity of the cervix between the modes of delivery and no difference in echogenicity of the vastus lateralis muscle between the modes of delivery was found.

Recovery after vaginal delivery

During vaginal delivery the levator ani muscle is stretched at least twice its initial length.[45,46] This stretch could lead to (micro) hematoma, edema, levator ani muscle avulsion and scar tissue formation as in all muscle tears. Therefore, damage to the levator ani muscle is an important risk factor for pelvic floor dysfunction.[47] The extent of the trauma and differences in trauma healing may be associated with the development of pelvic floor disorders. Little is known about the normal recovery process of the puborectalis muscle after vaginal delivery. Knowledge about the functionality of the puborectalis muscle after delivery may help us understanding the process of recovery and development of pelvic floor disorders. Since symptoms of pelvic floor disorders often appear years after delivery, our ultimate aim is to improve the recovery process shortly after delivery. This can only be done by using surrogate markers that reflect the extent of trauma and recovery process. With transperineal ultrasound examinations we studied the levator hiatal dimensions and MEP repeatedly between 1 day and 24 weeks after first vaginal delivery, and compared it to 12 weeks' gestation, in order to gain knowledge of the structural function.

We demonstrated that the recovery of the hiatal dimensions at rest and contraction, back to early pregnancy values, was completed after the first 3 weeks after vaginal delivery. In a magnetic resonance imaging (MRI) study of Tunn *et al.*, the area of the levator hiatus at rest of six women after delivery was measured 5 times between 1 day and 6 months postpartum and they showed that recovery took place within the first 2 weeks.[48] This is comparable to our finding. We also measured contractility, which is the difference between rest and contraction. Recovery of the contractility to early pregnancy state occurred between 6 and 12 weeks after delivery. In accordance to our finding, van Veelen *et al.* found that contractility at 6 months after delivery was comparable to early gestation.[6] The recovery of the contractile function is also in accordance with studies on strain injury in skeletal muscle in athletes, that show a recovery and return-to-play time comparable to our findings, ranging from 12 to 63 days.[49-51]

The hiatal area on maximum Valsalva maneuver and the difference between rest and Valsalva, distensibility, remained increased during the complete follow up period of 24 weeks. The persistent enlargement of the hiatal area on Valsalva after vaginal delivery was also demonstrated in several other studies.[6,12,52,53] Since the boundaries of the genital hiatus are determined by the puborectalis muscle, changes in the levator hiatal area are a reflection of changes in the puborectalis muscle itself. The persistent enlargement of the hiatal area on Valsalva and remaining increase in distensibility implicate that there are persistent structural changes in the puborectalis muscle after delivery, and that the support the puborectalis muscle offers to the pelvic organs is permanently compromised.[52,53] Therefore, understanding the structural composition of the puborectalis muscle, by the use of echogenicity measurements, will add to our understanding of the mechanisms of contraction and distension of the pelvic floor, and may help us understanding why functional recovery of the distensibility does not occur. The MEP, as a marker for structural changes, is significantly lower directly after delivery compared to the MEP during pregnancy, slowly increasing up to 24 weeks after delivery, but not reaching pregnancy values. The sharp decrease in MEP directly after delivery is most likely the resultant of edema and (micro)hematoma due to stretch trauma after delivery, since fluid appears dark on the ultrasound image.[54-56] Over time, in the interval of 24 weeks after delivery (from 6 weeks onward), the echogenicity increases and stabilizes, most likely due to the disappearance of hematoma and edema and the formation of scar tissue that appears bright on the ultrasound image.[62] The slowly increasing MEP during the recovery process is comparable to the general repair process phases of skeletal muscles; hematoma, scar tissue and recovery of function.[57-62] Since recovery of contractility to early pregnancy state occurred between 6 and 12 weeks and that the MEP stabilizes in this period, we hypothesize that the active support the puborectalis muscle offers to the pelvic floor organs (ability to contract) is recovered. We hypothesize that the persistent increase in distensibility, in combination with the lower MEP after delivery, is the resultant of a suboptimal collagen organization during the healing process after delivery. Another explanation could be that the ECM/collagen content remains decreased as compared to the pre-pregnancy status. Unfortunately, echogenicity values before pregnancy are not available to test this last hypothesis. Since ECM and collagen play an important role in this passive support the pelvic floor offers, a diminished collagen content would indicate a decrease in supportive capability, e.g. easier distensibility.

Based on our findings we have started a study in which we compare the recovery pattern of women with a primary CS to those who had a vaginal delivery.

General conclusion

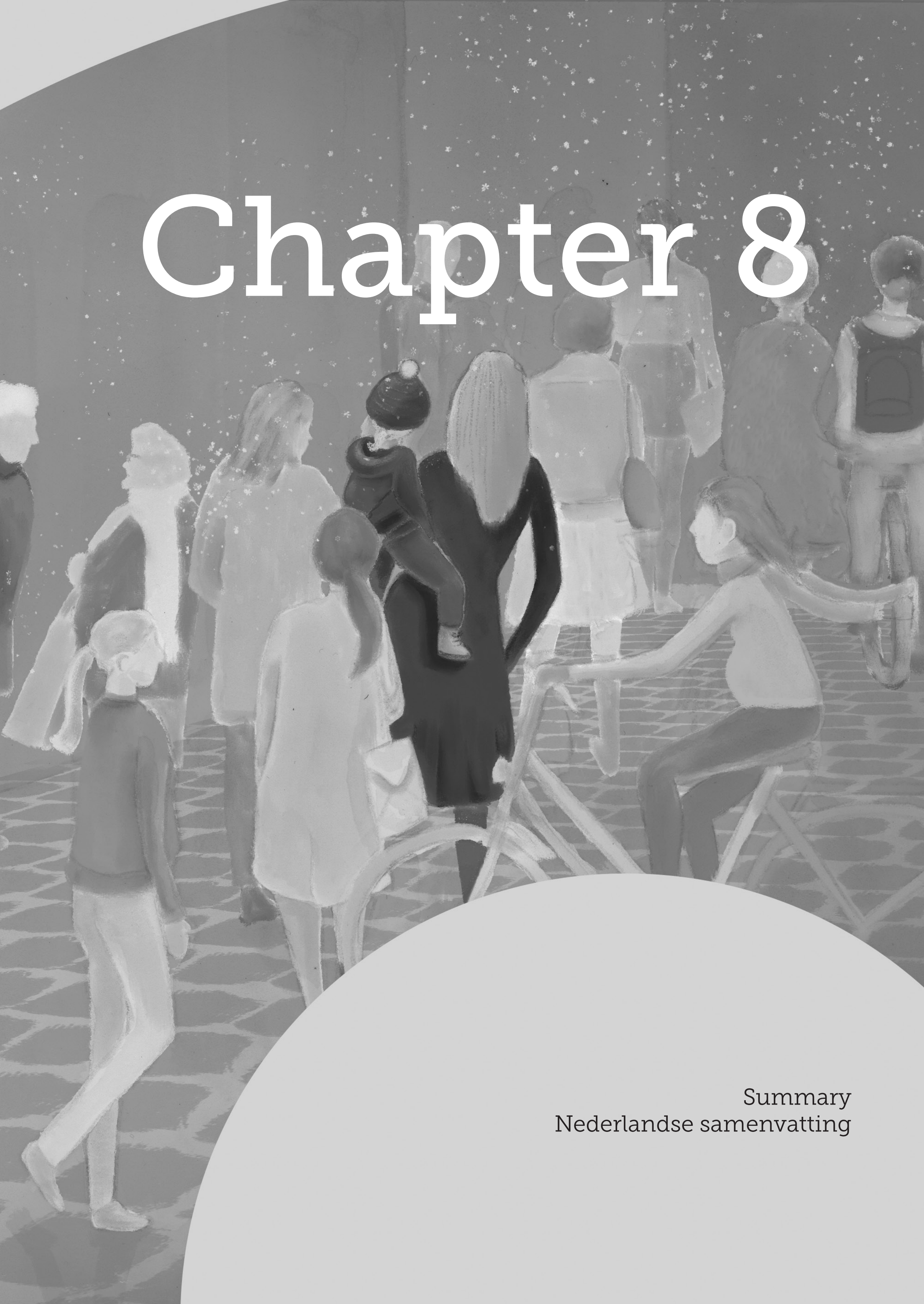
With the main findings of the various studies represented in this thesis we provided more insight in the structural composition of the pelvic floor during pregnancy and after delivery, its relation to SUI and its recovery in the first months after vaginal delivery by the use of 3D/4D transperineal ultrasound measurements. Our results are the first step towards developing a more functional based profile of the pelvic floor instead of looking only at pure anatomical landmarks. Pregnancy offers a unique opportunity to study the effect of trauma to the pelvic floor, and its healing. Understanding these mechanisms may help us selecting new treatment targets. In addition, pelvic floor ultrasound can also be used as a marker for recovery. New ultrasound software techniques like; strain measurement; automated segmentation by means of developing deep learning algorithms; real time 3 and 4D reconstruction, all are promising and bring us closer to our goal: the optimization of pelvic floor recovery after delivery.

References

1. MacLennan AH, Taylor AW, Wilson DH, Wilson D. The prevalence of pelvic floor disorders and their relationship to gender, age, parity and mode of delivery. *BJOG* 2000; 107 : 1460-1470.
2. Nygaard I. Pelvic floor recovery after childbirth. *Obstet Gynecol* 2015; 125 : 529-530.
3. McGuire EJ, Lytton B, Pepe V, Kohorn EI. Stress Urinary Incontinence. *Obstet Gynecol* 1976; 47 : 255-264.
4. Dietz HP. Ultrasound imaging of the pelvic floor. Part II: three-dimensional or volume imaging. *Ultrasound Obstet Gynecol* 2004; 23 : 615-625.
5. Cosimato C, Cipullo LM, Troisi J, Di Spiezio Sardo A, Tommaselli GA, Oro RR, Zullo F, Altieri V, Guida M. Ultrasonographic evaluation of urethrovesical junction mobility: correlation with type of delivery and stress urinary incontinence. *Int Urogynecol J* 2015; 26 : 1495-1502.
6. Van Veelen GA, Schweitzer KJ, van der Vaart CH. Ultrasound imaging of the pelvic floor: changes in anatomy during and after first pregnancy. *Ultrasound Obstet Gynecol* 2014; 44 : 476-480.
7. Falkert A, Willmann A, Endress E, Meint P, Seelbach-Gobel B. Three-dimensional ultrasound of pelvic floor: is there a correlation with delivery mode and persisting pelvic floor disorders 18-24 months after first delivery? *Ultrasound Obstet Gynecol* 2013; 41 : 204-209.
8. Wijma J, Weis Potters AE, de Wolf BT, Tinga DJ, Aarnoudse JG. Anatomical and functional changes in the lower urinary tract during pregnancy. *BJOG* 2001; 108 : 726-732.
9. Jundt K, Scheer I, Schiessl B, Karl K, Friese K, Peschers UM. Incontinence, bladder neck mobility, and sphincter ruptures in primiparous women. *Eur J Med Res* 2010; 15 : 246-252.
10. Shek KL, Kruger J, Dietz HP. The effect of pregnancy on hiatal dimensions and urethral mobility: an observational study. *Int Urogynecol J* 2012; 23 : 1561-1567.
11. DeLancey JO, Miller JM, Kearney R, Howard D, Reddy P, Umek W, Guire KE, Margulies RU, Ashton-Miller JA. Vaginal birth and de novo stress incontinence: relative contributions of urethral dysfunction and mobility. *Obstet Gynecol* 2007; 110 : 354-362.
12. Chan SS, Cheung RY, Yiu KW, Lee LL, Leung TY, Chung TK. Pelvic floor biometry during a first singleton pregnancy and the relationship with symptoms of pelvic floor disorders: a prospective observational study. *BJOG* 2014; 121 : 121-129.
13. Ashton-Miller JA, DeLancey JO. Functional anatomy of the female pelvic floor. *Ann N Y Acad Sci* 2007; 1101 : 266-296.
14. Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *N Engl J Med* 2014; 371 : 2237-2238.
15. Alperin M, Kaddis T, Pichika R, Esparza MC, Lieber RL. Pregnancy-induced adaptations in intramuscular extracellular matrix of rat pelvic floor muscles. *Am J Obstet Gynecol* 2016; 215 : 210.e1-210.e7.
16. Abeysekera MV, Morris JA, Davis GK, O'Sullivan AJ. Alterations in energy homeostasis to favour adipose tissue gain: A longitudinal study in healthy pregnant women. *Aust N Z J Obstet Gynaecol* 2016; 56 : 42-48.
17. Meyer S, Bachelard O, De Grandi P. Do bladder neck mobility and urethral sphincter function differ during pregnancy compared with during the non-pregnant state? *Int Urogynecol J Pelvic Floor Dysfunct* 1998; 9 : 397-404.
18. Athanasiou S, Khullar V, Boos K, Salvatore S, Cardozo L. Imaging the urethral sphincter with three-dimensional ultrasound. *Obstet Gynecol* 1999; 94 : 295-301.
19. Kondo Y, Homma Y, Takahashi S, Kitamura T, Kawabe K. Transvaginal ultrasound of urethral sphincter at the mid urethra in continent and incontinent women. *J Urol* 2001; 165 : 149-152.

20. Digesu GA, Calandrini N, Derpapas A, Gallo P, Ahmed S, Khullar V. Intraobserver and interobserver reliability of the three-dimensional ultrasound imaging of female urethral sphincter using a translabial technique. *Int Urogynecol J* 2012; 23 : 1063-1068.
21. Van Veelen A, Schweitzer K, van der Vaart H. Ultrasound assessment of urethral support in women with stress urinary incontinence during and after first pregnancy. *Obstet Gynecol* 2014; 124 : 249-256.
22. Lee JC, Healy J. Sonography of lower limb muscle injury. *AJR Am J Roentgenol* 2004; 182 : 341-351.
23. Vanhoenacker FM, Baert AL, Faletti C, Maas M, Gielen JL. *Imaging of Orthopedic Sports Injuries*. Springer Berlin Heidelberg, 2007.
24. Ashcroft GS, Ashworth JJ. Potential role of estrogens in wound healing. *Am J Clin Dermatol* 2003; 4 : 737-743.
25. Hausswirth C, Le Meur Y. Physiological and nutritional aspects of post-exercise recovery: specific recommendations for female athletes. *Sports Med* 2011; 41 : 861-882.
26. Tiidus PM, Deller M, Bombardier E, Gul M, Liu XL. Estrogen supplementation failed to attenuate biochemical indices of neutrophil infiltration or damage in rat skeletal muscles following ischemia. *Biol Res* 2005; 38 : 213-223.
27. Kwee A, Elferink-Stinkens PM, Reuwer PJ, Bruinse HW. Trends in obstetric interventions in the Dutch obstetrical care system in the period 1993-2002. *Eur J Obstet Gynecol Reprod Biol* 2007; 132 : 70-75.
28. Reuwer PJ, Bruinse HW, Franx A. *Proactive support of labour*. Cambridge university press, 2015.
29. Kozinszky Z, Orvos H, Zoboki T, Katona M, Wayda K, Pal A, Kovacs L. Risk factors for cesarean section of primiparous women aged over 35 years. *Acta Obstet Gynecol Scand* 2002; 81 : 313-316.
30. Usha Kiran TS, Hemmadi S, Bethel J, Evans J. Outcome of pregnancy in a woman with an increased body mass index. *BJOG* 2005; 112 : 768-772.
31. Fitzpatrick M, McQuillan K, O'Herlihy C. Influence of persistent occiput posterior position on delivery outcome. *Obstet Gynecol* 2001; 98 : 1027-1031.
32. Morris JM, Thompson K, Smithey J, Gaffney G, Cooke I, Chamberlain P, Hope P, Altman D, MacKenzie IZ. The usefulness of ultrasound assessment of amniotic fluid in predicting adverse outcome in prolonged pregnancy: a prospective blinded observational study. *BJOG* 2003; 110 : 989-994.
33. Saunders N, Amis S, Marsh M. The prognostic value of fetal ultrasonography before induction of labour. *Br J Obstet Gynaecol* 1992; 99 : 903-906.
34. Schuit E, Kwee A, Westerhuis ME, Van Dessel HJ, Graziosi GC, Van Lith JM, Nijhuis JG, Oei SG, Oosterbaan HP, Schuitemaker NW, Wouters MG, Visser GH, Mol BW, Moons KG, Groenwold RH. A clinical prediction model to assess the risk of operative delivery. *BJOG* 2012; 119 : 915-923.
35. Peregrine E, O'Brien P, Omar R, Jauniaux E. Clinical and ultrasound parameters to predict the risk of cesarean delivery after induction of labor. *Obstet Gynecol* 2006; 107 : 227-233.
36. Chen G, Uryasev S, Young TK. On prediction of the cesarean delivery risk in a large private practice. *Am J Obstet Gynecol* 2004; 191 : 616-24; discussion 624-5.
37. Harper DM, Johnson CA, Harper WH, Liese BS. Prenatal predictors of cesarean section due to labor arrest. *Arch Gynecol Obstet* 1995; 256 : 67-74.
38. Hueston WJ. Development of a cesarean delivery risk score. *Obstet Gynecol* 1994; 84 : 965-968.
39. Roman H, Blondel B, Breart G, Goffinet F. Do risk factors for elective cesarean section differ from those of cesarean section during labor in low risk pregnancies? *J Perinat Med* 2008; 36 : 297-305.
40. Seshadri L, Mukherjee B. A predictive model for cesarean section in low risk pregnancies. *Int J Gynaecol Obstet* 2005; 89 : 94-98.
41. Van Veelen GA, Schweitzer KJ, van Hoogenhuijze NE, van der Vaart CH. Association between levator hiatal dimensions on ultrasound during first pregnancy and mode of delivery. *Ultrasound Obstet Gynecol* 2015; 45 : 333-338.

42. Grob AT, Withagen MI, van de Waarsenburg MK, Schweitzer KJ, van der Vaart CH. Association of First-Trimester Echogenicity of the Puborectalis Muscle With Mode of Delivery. *Obstet Gynecol* 2016; 127 : 1021-1026.
43. Alperin M, Lawley DM, Esparza MC, Lieber RL. Pregnancy-induced adaptations in the intrinsic structure of rat pelvic floor muscles. *Am J Obstet Gynecol* 2015; 213 : 191.e1-191.e7.
44. Alperin M, Lawley DM, Esparza MC, Lieber RL. Pregnancy-induced adaptations in the intrinsic structure of rat pelvic floor muscles. *Am J Obstet Gynecol* 2015; 213 : 191.e1-191.e7.
45. Lien KC, Mooney B, DeLancey JO, Ashton-Miller JA. Levator ani muscle stretch induced by simulated vaginal birth. *Obstet Gynecol* 2004; 103 : 31-40.
46. Sindhwani N, Bamberg C, Famaey N, Callewaert G, Dudenhausen JW, Teichgraber U, Deprest J. In vivo evidence of significant levator ani muscle stretch on MR images of a live childbirth. *Am J Obstet Gynecol* 2017; 217 : 194.e1-194.e8.
47. Van Delft K, Thakar R, Shobeiri SA, Sultan AH. Levator hematoma at the attachment zone as an early marker for levator ani muscle avulsion. *Ultrasound Obstet Gynecol* 2014; 43 : 210-217.
48. Tunn R, DeLancey JO, Howard D, Thorp JM, Ashton-Miller JA, Quint LE. MR imaging of levator ani muscle recovery following vaginal delivery. *Int Urogynecol J Pelvic Floor Dysfunct* 1999; 10 : 300-307.
49. Lovering RM, De Deyne PG. Contractile function, sarcolemma integrity, and the loss of dystrophin after skeletal muscle eccentric contraction-induced injury. *Am J Physiol Cell Physiol* 2004; 286 : C230-8.
50. Lovering RM, Roche JA, Bloch RJ, De Deyne PG. Recovery of function in skeletal muscle following 2 different contraction-induced injuries. *Arch Phys Med Rehabil* 2007; 88 : 617-625.
51. Hickey JT, Timmins RG, Maniar N, Williams MD, Opar DA. Criteria for Progressing Rehabilitation and Determining Return-to-Play Clearance Following Hamstring Strain Injury: A Systematic Review. *Sports Med* 2016; .
52. Shek KL, Dietz HP. Intrapartum risk factors for levator trauma. *BJOG* 2010; 117 : 1485-1492.
53. Staer-Jensen J, Siafarikas F, Hilde G, Benth JS, Bo K, Engh ME. Postpartum recovery of levator hiatus and bladder neck mobility in relation to pregnancy. *Obstet Gynecol* 2015; 125 : 531-539.
54. Kullmer K, Sievers KW, Rompe JD, Nagele M, Harland U. Sonography and MRI of experimental muscle injuries. *Arch Orthop Trauma Surg* 1997; 116 : 357-361.
55. Mattiussi G, Baldassi PT, Pasta G, Burani A, Moreno C. Perivascular Adductor Longus muscle injury: Ultrasound and Magnetic Resonance Imaging findings. *Muscles Ligaments Tendons J* 2017; 7 : 376-387.
56. Peixinho CC, Ribeiro MB, Resende CM, Werneck-de-Castro JP, de Oliveira LF, Machado JC. Ultrasound biomicroscopy for biomechanical characterization of healthy and injured triceps surae of rats. *J Exp Biol* 2011; 214 : 3880-3886.
57. Cannon JG, St Pierre BA. Cytokines in exertion-induced skeletal muscle injury. *Mol Cell Biochem* 1998; 179 : 159-167.
58. Jarvinen TA, Jarvinen TL, Kaariainen M, Kalimo H, Jarvinen M. Muscle injuries: biology and treatment. *Am J Sports Med* 2005; 33 : 745-764.
59. Kaariainen M, Jarvinen T, Jarvinen M, Rantanen J, Kalimo H. Relation between myofibers and connective tissue during muscle injury repair. *Scand J Med Sci Sports* 2000; 10 : 332-337.
60. Fernandes TL, Pedrinelli A, Hernandez AJ. Muscle Injury - Physiopathology, Diagnosis, Treatment and Clinical Presentation. *Rev Bras Ortop* 2015; 46 : 247-255.
61. Kaariainen M, Kaariainen J, Jarvinen TL, Sievanen H, Kalimo H, Jarvinen M. Correlation between biomechanical and structural changes during the regeneration of skeletal muscle after laceration injury. *J Orthop Res* 1998; 16 : 197-206.
62. Dimpfl T, Jaeger C, Mueller-Felber W, Anthuber C, Hirsch A, Brandmaier R, Schuessler B. Myogenic changes of the levator ani muscle in premenopausal women: the impact of vaginal delivery and age. *Neurourol Urodyn* 1998; 17 : 197-205.



Chapter 8

Summary
Nederlandse samenvatting

Summary

Chapter 1

The pelvic floor provides support to the pelvic organs. The most important muscle of the pelvic floor is the levator ani muscle complex that encloses the genital hiatus through which urethra, vagina and rectum pass.

Pregnancy and childbirth are strongly associated with pelvic floor dysfunction, leading to pelvic floor disorders such as pelvic organ prolapse and urinary incontinence. These disorders are highly prevalent conditions with a negative impact on quality of life. Although there is a clear association between pregnancy and childbirth and pelvic floor disorders, the exact mechanism is complex. By the use of 3D transperineal ultrasound we are able to visualize the puborectalis muscle, which is part of the levator ani muscle complex. This visualization permits reliable assessment of hiatal dimensions, echogenicity and area of the puborectalis muscle. Several previous studies used transperineal ultrasound to assess changes in pelvic floor anatomy during pregnancy and after delivery. Changes in the structural composition of the pelvic floor during pregnancy and after delivery, and the normal recovery after pregnancy and vaginal delivery are largely unknown and represented the core focus of the current thesis.

Chapter 2

One of the pelvic floor disorders is stress urinary incontinence (SUI). The most common finding in women with SUI is hypermobility of the proximal urethra during increased intra-abdominal pressure, resulting in leakage of urine. Normal urethral function depends not only on urethral support but also on the internal urethral closure mechanism. In contrast to studies on bladder neck and urethral mobility, little is known about possible changes of the urethral sphincter muscle during pregnancy. Therefore, the aim of our study was to assess urethral sphincter echogenicity during and after pregnancy and its association with SUI symptoms. After establishing that the method we developed showed a good intra- and interobserver reliability, we studied the changes during and after pregnancy. The mid-urethral mean echogenicity of our study group with the pelvic floor at rest was significantly higher during pregnancy as compared to 6 months after delivery. The echogenicity of the mid-urethra during a pelvic floor contraction did not differ between pregnancy and postpartum measurements. The most likely explanation for the higher echogenicity during pregnancy is related to the fact that pregnancy, with its increased levels of progesterone, induces an increase in fat storage and collagen in muscle tissue. Both will increase echogenicity as compared to non-pregnant values.

8

Chapter 3

The puborectalis muscle encloses the hiatal area. During pregnancy SUI has been associated with the width of the hiatal area. The observation that during pregnancy a large hiatal area is associated with SUI raises the question if this is related to structural abnormalities of the puborectalis muscle. We set out to assess the association between the mean echogenicity of the puborectalis muscle (MEP) and SUI symptoms during and after first pregnancy. We were able to show that women with SUI after delivery had a statistically significant higher MEP as compared to continent women. However, the effect size of this finding was small (0.30). This indicates that the ratio between muscle cells and extra cellular matrix (ECM) shifted towards the ECM in women with SUI. Literature on muscle injuries shows that scar tissue formation, which will represent itself as a higher echogenicity, is associated with a loss of contractility function of the muscle. If the increase in ECM in the puborectalis muscle indeed indicates that this will compromise its contractile function, its contribution to the urethral support may be compromised and hypermobility of the urethra occurs. No differences in the MEP between women with and without SUI during pregnancy were found. We hypothesize that this lack of difference is based on the fact that injury due to delivery has not yet occurred. Apparently, the mechanism of developing SUI during pregnancy is different from the mechanism of developing SUI after childbirth.

Chapter 4

Previously we studied the possible association between ultrasound parameters of the pelvic floor during pregnancy and mode of delivery. Hiatal dimensions and MEP were statistically significant different at 12 weeks gestation in women who finally delivered by a caesarean section (CS) due to failure to progress (FTP) compared to women that delivered vaginally. It has to be noted that the number of women with a CS due to FTP in this single center study was small and that the difference in MEP was only observed during contraction. Therefore, we set out to study these findings in a multicenter study.

Since the effect of pregnancy on muscle tissue composition may also differ between the pelvic floor and other muscles, we also assessed the association between the echogenicity of the cervix and the vastus lateralis muscle and mode of delivery.

We were unable to confirm the previous results of a smaller MEP and hiatal transverse diameter and area during contraction at 12 weeks' pregnancy in women with a CS due to FTP. In addition, no association between the echogenicity of the cervix and vastus lateralis muscle and mode of delivery was found. Possible explanations for the lack of difference in the different modes of delivery of this study were considered but no explanation was found. We did find a mean 20 points lower MEP in all patients compared to the previous study despite the use of the same ultrasound equipment and settings. The mean lower MEP was explainable due to the higher depth in the ultrasound images of the recent study. However, since we observed a lower MEP in all groups we do not think this may have affected the comparison between groups.

Chapter 5

Trauma during delivery is known as one of the most important causes of pelvic floor disorders. The extent of the damage that occurs during delivery and/or differences in the recovery process may be related to the development of pelvic floor disorders. Little is known about normal recovery of the pelvic floor in the first weeks after vaginal delivery. In particular, there is a paucity of longitudinal data on this recovery. A better understanding of the recovery of the pelvic floor after vaginal delivery is of importance when considering early interventions improving recovery with for example stem cells and its culture medium. With transperineal ultrasound examinations we studied the levator hiatal dimensions repeatedly between 1 day and 24 weeks after first vaginal delivery, and compared it to 12 weeks' gestation. We demonstrated that the recovery of the hiatal dimensions at rest and contraction, back to early pregnancy values, was completed within the first 3 weeks after vaginal delivery. We also measured contractility, which is the difference between rest and contraction. Recovery of the contractility to early pregnancy state occurred between 6 and 12 weeks after delivery. This recovery is comparable to existing research on the pelvic floor and skeletal muscles. The hiatal area on maximum Valsalva maneuver and the difference between rest and Valsalva, distensibility, remained increased during the complete follow up period of 24 weeks. The persistent enlargement of the hiatal area on Valsalva after vaginal delivery was also demonstrated in several other studies.

Chapter 6

Persistent enlargement of the hiatal area on Valsalva and increased distensibility 24 weeks after delivery, implicate that there are persistent structural changes in the puborectalis muscle after delivery. Therefore, structural composition of the puborectalis muscle, by the use of echogenicity measurements, was studied. The MEP is significantly lower directly after delivery compared to the MEP during pregnancy, slowly increasing up to 24 weeks after delivery, but not reaching pregnancy values. The sharp decrease in MEP directly after delivery is most likely the resultant of edema and (micro)hematoma due to stretch trauma after delivery, since fluid appears dark on the ultrasound image. Over time, in the interval of 24 weeks after delivery (from 6 weeks onward), the echogenicity increases and stabilizes, most likely due to the disappearance of edema and hematoma and the formation of scar tissue that appears bright on the ultrasound image. The slowly increasing MEP during the recovery process is comparable to the general repair process phases of skeletal muscles; hematoma, scar tissue and recovery of function. Since recovery of contractility to early pregnancy state occurred between 6 and 12 weeks and that the MEP stabilizes in this period, we hypothesize that the active support the puborectalis muscle offers to the pelvic floor organs (ability to contract) is recovered. We hypothesize that the persistent increase in distensibility, in combination with the lower MEP after delivery, is the resultant of a suboptimal collagen organization during the healing process after delivery. Another explanation could be that the ECM/collagen content remains decreased as compared to the pre-pregnancy status. Since ECM and collagen play an important role in this passive support the pelvic floor offers, a diminished collagen content would indicate a decrease in supportive capability, e.g. easier distensibility.

Chapter 7

In this chapter we summarize the interpretation of the main findings within this thesis, we consider potential implications for clinical practice and propose recommendations for future research. With the main findings of the various studies represented in this thesis we provided more insight in the structural composition of the pelvic floor during pregnancy and after delivery, its relation to SUI and its recovery in the first months after vaginal delivery by the use of 3D/4D transperineal ultrasound measurements. Our results are the first step towards developing a more functional based profile of the pelvic floor instead of looking only at anatomical landmarks. Understanding the extent of trauma and mechanisms of healing may help us selecting new treatment targets after pregnancy and delivery. In addition, pelvic floor ultrasound can also be used as a marker for recovery. New ultrasound software techniques like; strain measurement; automated segmentation by means of developing deep learning algorithms; real time 3 and 4D reconstruction, all are promising and bring us closer to our goal: the optimal recovery of the pelvic floor after delivery.

8

Nederlandse samenvatting

Hoofdstuk 1

De bekkenbodemspieren vormen de ondersteuning van de bekkenorganen. De belangrijkste spier van de bekkenbodem is de levator ani spier die de levator hiatus omsluit. Door de levator hiatus passeren de urethra, vagina en het rectum.

Zwangerschap en bevalling zijn sterk geassocieerd met het disfunctioneren van de bekkenbodem wat leidt tot bekkenbodemproblemen zoals verzakking en incontinentie. Deze problemen komen veel voor en hebben een negatieve impact op de kwaliteit van leven. Hoewel er een duidelijke associatie is tussen zwangerschap en bevalling en bekkenbodemproblemen, is het onderliggende mechanisme niet geheel opgehelderd. Door het gebruik van 3D transperineale echografie zijn we in staat om de puborectalis spier, die onderdeel is van de levator ani, in beeld te brengen. Met deze beeldvorming kunnen we betrouwbare metingen van de levator hiatus, echogeniciteit en oppervlakte van de puborectalis spier doen. Transperineale echografie wordt reeds veelvuldig gebruikt om veranderingen in de bekkenbodemanatomie tijdens zwangerschap en bevalling te beoordelen. Veranderingen in de structurele compositie van de bekkenbodem tijdens zwangerschap en na bevalling, en normaal herstel na zwangerschap en bevalling zijn echter nog onbekend en worden in dit proefschrift geëxploreerd.

Hoofdstuk 2

Een van de meest voorkomende bekkenbodemklachten is stress urine-incontinentie (SUI). De belangrijkste bevinding bij vrouwen met SUI is hypermobiliteit van de urethra tijdens druk verhogende momenten, wat resulteert in urineverlies. Het functioneren van de urethra is afhankelijk van de urethrale support maar ook van het intrinsieke sluitingsmechanisme. In tegenstelling tot studies naar blaashals en urethra mobiliteit, is er weinig bekend over mogelijke veranderingen van de urethrale sfincter tijdens zwangerschap. Daarom was het doel van onze studie om de echogeniciteit van de urethrale sfincter en de associatie met SUI-symptomen tijdens en na de zwangerschap te bepalen. Na het vaststellen van de betrouwbaarheid van de onderzoeksmethode (bij meerdere metingen door dezelfde onderzoeker en bij metingen door verschillende onderzoekers) hebben we gekeken naar de veranderingen tijdens en na zwangerschap. De mid-urethrale gemiddelde echogeniciteit van onze studiegroep terwijl de bekkenbodem in rust was, was significant hoger tijdens de zwangerschap vergeleken met 6 maanden na de bevalling. De echogeniciteit van de mid-urethra tijdens contractie van de bekkenbodem was niet verschillend tussen zwangerschap en na bevalling. De meest waarschijnlijke oorzaak van de hogere echogeniciteit tijdens de zwangerschap is gerelateerd aan het feit dat zwangerschap, met gestegen progesteron levels, een toename in vetopslag en collageen in spierweefsel induceert. Beiden zullen de echogeniciteit verhogen vergeleken met niet-zwangere waarden.

Hoofdstuk 3

De puborectalis spier omsluit de levator hiatus. Tijdens zwangerschap is SUI geassocieerd met de breedte van de levator hiatus. Omdat een grotere hiatale oppervlakte tijdens zwangerschap geassocieerd is met SUI rees de vraag of SUI gerelateerd is aan structurele afwijkingen van de puborectalis spier. We hebben de associatie tussen de gemiddelde echogeniciteit van de puborectalis spier (MEP) en SUI-symptomen tijdens en na een eerste bevalling onderzocht. We zagen dat vrouwen met SUI na de bevalling een statistisch significant hogere MEP hadden vergeleken met continente vrouwen. De effect size was echter klein (0.30). Dit impliceert dat de ratio tussen spiercellen en extracellulaire matrix (ECM) is verschoven richting ECM bij vrouwen met SUI. In literatuur over spierschade wordt gezien dat littekenvorming, dat gepaard gaat met een hogere echogeniciteit, geassocieerd is met een verlies van contractiliteit van een spier. Als de toename van ECM in de puborectalis spier inderdaad impliceert dat dit de contractiliteit doet verminderen, dan zou de urethrale support verminderd zijn waardoor hypermobiliteit van de urethra ontstaat. Er is geen verschil gevonden tussen de MEP van vrouwen met en zonder SUI tijdens de zwangerschap. Onze hypothese is dat dit komt omdat er nog geen bevalschade is opgetreden. Blijkbaar is het mechanisme van het ontwikkelen van SUI tijdens de zwangerschap anders dan na de bevalling.

Hoofdstuk 4

In eerdere onderzoeken is gekeken naar de mogelijke associatie tussen echo parameters van de bekkenbodem tijdens zwangerschap en de manier van bevallen. Hiatale dimensies (de oppervlakte, breedte en hoogte van de levator hiatus) en MEP waren significant verschillend bij 12 weken zwangerschap bij vrouwen die uiteindelijk een secundaire keizersnede vanwege het niet vorderen van de ontsluiting of uitdrijving ondergingen, vergeleken met vrouwen die vaginaal bevielen. Er waren echter maar weinig vrouwen in deze groep, de studie werd in maar één centrum uitgevoerd en de bevindingen werden alleen gevonden met de bekkenbodem in contractie. Daarom hebben we een multicenter studie opgezet om hier uitgebreider naar te kijken.

Omdat het effect van zwangerschap op de samenstelling van spierweefsel anders kan zijn tussen de bekkenbodemspieren en spieren elders in het lichaam, hebben we ook gekeken naar de associatie tussen de echogeniciteit van de baarmoedermond en de vastus lateralis spier in het been en de manier van bevallen.

We konden de bevindingen uit eerdere studies, namelijk een lagere MEP en kleinere transversale diameter en oppervlakte van de levator hiatus tijdens contractie bij 12 weken zwangerschap bij vrouwen die bevielen middels een secundaire keizersnede vanwege het niet vorderen van de ontsluiting of uitdrijving, niet bevestigen. Aanvullend was er geen verschil tussen de echogeniciteit van de baarmoedermond en de echogeniciteit van de vastus lateralis spier en de manier van bevallen. Verschillende verklaringen voor het ontbreken van een verschil in de manier van bevallen werden overwogen maar een passende verklaring werd niet gevonden. We vonden wel een lagere MEP van 20 punten in alle patiënten vergeleken met de eerdere studie desondanks dezelfde apparatuur en instellingen. Deze lagere MEP kan verklaard worden door een verschil in de diepte waarop gemeten werd tussen de voorgaande en de recente studie. Maar omdat we het verschil in alle groepen vonden denken we dat dit de vergelijking tussen de groepen niet heeft beïnvloed.

Hoofdstuk 5

Het is bekend dat schade ten tijde van de bevalling een van de meest belangrijke oorzaken is van bekkenbodemproblemen. De mate van deze schade en/of de verschillen in het herstelproces zijn mogelijk gerelateerd aan het ontstaan van bekkenbodemproblematiek. Er is weinig bekend over het normale herstel van de bekkenbodem in de eerste weken na een vaginale baring. In het bijzonder is er weinig longitudinale data over het herstel. Een beter begrip van het herstel van de bekkenbodem na een vaginale baring is van belang als vroege herstel-bevorderende interventies worden overwogen zoals het gebruik van stamcellen en hun kweekmedium bij vrouwen bij wie het herstel afwijkend is. Daarom hebben we met transperineale echografie herhaaldelijk gekeken naar de hiatale dimensies tussen 1 dag en 24 weken na de eerste vaginale baring. Dit hebben we steeds vergeleken met de beelden van 12 weken zwangerschap. We zagen dat de hiatale dimensies, met de bekkenbodem in rust en contractie, vergelijkbaar waren met 12 weken zwangerschap binnen 3 weken na de vaginale baring. We hebben ook gekeken naar contractiliteit, het verschil tussen rust en contractie. Hierbij zagen we dat de contractiliteit vergelijkbaar was met 12 weken zwangerschap tussen 6 en 12 weken na de bevalling. Dit herstel is vergelijkbaar met andere studies die hebben gekeken naar de bekkenbodem maar ook met studies die keken naar het herstel van skeletspieren. De hiatale oppervlakte tijdens maximale Valsalva en het verschil tussen rust en Valsalva, distensibiliteit, bleven toegenomen ten tijde van de follow-up periode van 24 weken. De persisterende toegenomen hiatale oppervlakte tijdens Valsalva na een vaginale baring werd in andere studies ook gezien.

Hoofdstuk 6

De toegenomen hiatale oppervlakte en distensibiliteit 24 weken na de bevalling impliceren dat er persisterende structurele veranderingen zijn in de puborectalis spier na de bevalling. Daarom hebben we de structurele compositie van de puborectalis spier middels

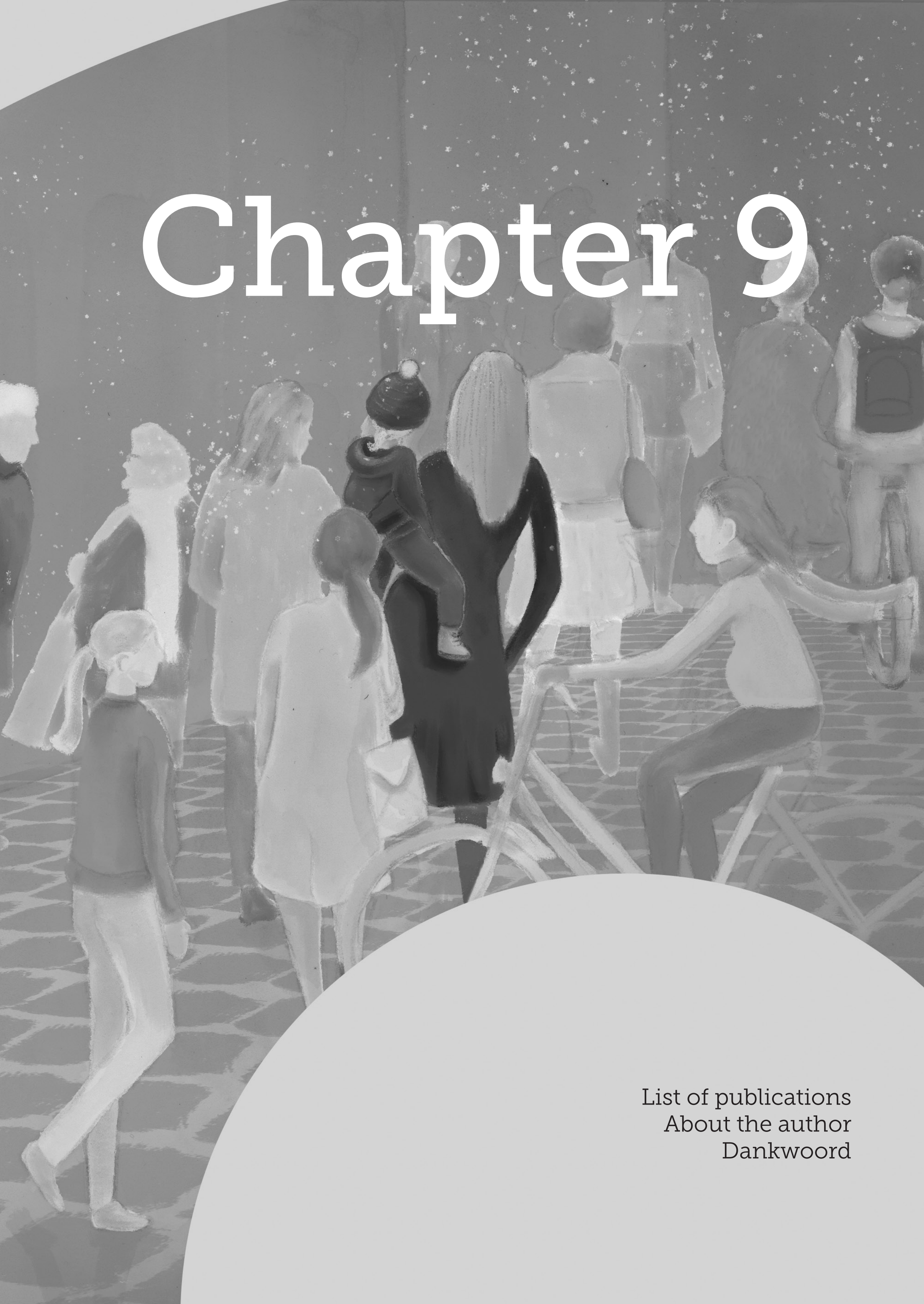
8

echogeniciteit onderzocht. De MEP is significant lager direct na de bevalling en stijgt langzaam in de postpartum periode, maar blijft lager vergeleken met de bevindingen bij 12 weken zwangerschap. De scherpe daling van de MEP direct na de bevalling wordt meest waarschijnlijk veroorzaakt door oedeem en (micro)hematomen (zwart op echobeeld) door schade door uitrekken tijdens de bevalling. Na verloop van tijd, vanaf 6 weken na de bevalling, stijgt en stabiliseert de echogeniciteit wat het meest past bij het verdwijnen van oedeem en hematomen en ontwikkeling van lettekenweefsel (wit op echobeeld). De langzaam stijgende MEP tijdens het herstelproces is vergelijkbaar met het herstelproces van skeletspieren; hematomen, littekenweefsel en herstel van functie. Omdat het herstel van de contractiliteit tussen 6 en 12 weken na de bevalling optreedt en dat de MEP op dat moment stabiliseert, hebben wij de hypothese dat de actieve ondersteuning die de puborectalis spier aan de bekkenorganen biedt (mogelijkheid tot samentrekken) is hersteld. We denken daarnaast dat de persisterend toegenomen distensibiliteit, in combinatie met een lagere MEP na de bevalling, het resultaat is van een suboptimale organisatie van collageen tijdens het helingsproces na de bevalling. Een andere verklaring zou kunnen zijn dat de ECM/collageen component verlaagd blijft vergeleken met voor de zwangerschap. Omdat ECM en collageen een belangrijke rol spelen in de passieve ondersteuning die de bekkenbodem aan de bekkenorganen verschaft, zou een verlaagde collageen component een verminderde ondersteuning bewerkstelligen, wat bijvoorbeeld kan leiden tot een toegenomen distensibiliteit.

Hoofdstuk 7

In dit hoofdstuk vatten we de interpretatie van de belangrijkste bevindingen in dit proefschrift samen, overwegen we potentiele consequenties voor de kliniek en doen we aanbevelingen voor toekomstig onderzoek.

Met de bevindingen van de verschillende studies in dit proefschrift geven we een beter inzicht in de structurele compositie van de bekkenbodem tijdens de zwangerschap en na de bevalling, de relatie met SUI en het herstel in de eerste maanden na een vaginale baring met behulp van 3D transperineale echografie. Onze resultaten zijn de eerste stap naar het ontwikkelen van een meer functiegericht profiel van de bekkenbodem in plaats van het exploreren van anatomische oriëntatiepunten. Begrip van de omvang van trauma en het mechanisme van herstel helpt ons bij het ontwikkelen van nieuwe therapie-doelen na zwangerschap en bevalling. Aanvullend kan bekkenbodem echografie gebruikt worden als marker voor herstel. Nieuwe echo softwaretechnieken, zoals strain metingen, automatische segmentatie door de ontwikkeling van deep learning en real time 3 en 4D reconstructie, zijn veelbelovend en brengen ons dichter bij ons doel: het optimale herstel van de bekkenbodem na de bevalling.



Chapter 9

List of publications
About the author
Dankwoord

List of publications

Van de Waarsenburg MK, Withagen MIJ, van den Noort F, Schagen van Leeuwen JH, van der Vaart CH. Echogenicity of the puborectalis muscle, the cervix and the vastus lateralis muscle in pregnancy in relation to mode of delivery. *Ultrasound Obstet Gynecol*. 2018.

Van de Waarsenburg MK, van der Vaart CH, Withagen MIJ. Structural changes in the puborectalis muscle after vaginal delivery. *Ultrasound Obstet Gynecol*. 2018 Jun 26. doi: 10.1002/uog.19170. [Epub ahead of print].

Van de Waarsenburg MK, van Hoogenhuijze NE, Grob AT, Schweitzer KJ, Withagen MI, van der Vaart CH. Method and reliability of mid-urethral area and echogenicity and changes during pregnancy and after delivery. *Int Urogynecol J*. 2018; 52 : 1379-1385.

Van de Waarsenburg MK, Verberne EA, van der Vaart CH, Withagen MI. Recovery of the puborectalis muscle after vaginal delivery: an ultrasound study. *Ultrasound Obstet Gynecol*. 2018; 52 : 390-395.

Grob AT, Hitschrich N, **van de Waarsenburg MK**, Withagen MI, Schweitzer KJ, van der Vaart CH. Changes in the global strain of the puborectalis muscle during pregnancy and postpartum. *Ultrasound Obstet Gynecol*. 2018; 51 : 537-542.

Grob AT, Withagen MI, **van de Waarsenburg MK**, Schweitzer KJ, van der Vaart CH. Association of First-Trimester Echogenicity of the Puborectalis Muscle With Mode of Delivery. *Obstet Gynecol*. 2016; 127 : 1021-1026.

Van de Waarsenburg MK, Withagen MI, Grob AT, Schweitzer KJ, van Veelen GA, van der Vaart CH. Mean echogenicity and area of puborectalis muscle in women with stress urinary incontinence during pregnancy and after delivery. *Int Urogynecol J*. 2016; 27 : 1723-1728.

Grob ATM, Withagen MIJ, **van de Waarsenburg MK**, Schweitzer KJ, van der Vaart CH. Changes in the mean echogenicity and area of the puborectalis muscle during pregnancy and postpartum. *Int Urogynecol J*. 2016; 27 : 895-901.

Van de Waarsenburg K, Vollebregt A, Milani F, Oude Rengerink K, van der Vaart H. Comments on Lone et al.: One-year prospective comparison of vaginal pessaries and surgery for pelvic organ prolapse using the validated ICIQ-VS and ICIQ-UI (SF) questionnaires. *Int Urogynecol J*. 2015; 26 : 1399.

Van de Waarsenburg MK, Hack KE, Rijpma RJ, Mulder EJ, Pistorius L, Derks JB. Ultrasonographic prediction of birth weight discordance in twin pregnancies. *Prenat Diagn.* 2015; 35 : 906-912.

Van Haastert IC, Groenendaal, **van de Waarsenburg MK**, Eijsermans MJC, Koopman-Esseboom C, Jongmans MJ, Helders PJM, de Vries LS. Active head lifting from supine in early infancy: an indicator for non-optimal cognitive outcome in late infancy. *Dev Med Child Neurol.* 2012; 54 : 538-543.

9

About the author

Kim Verheijen – van de Waarsenburg werd op 9 november 1985 geboren in het St. Lambertus ziekenhuis in Helmond. In 2004 behaalde zij haar VWO-diploma aan het Carolus Borromeus College te Helmond. Hierna heeft zij, nadat zij werd uitgeloot voor de opleiding Geneeskunde, gedurende een jaar de opleiding Psychologie gevolgd aan de Universiteit van Utrecht. In het jaar daarna werd zij ingeloot voor de opleiding Geneeskunde te Utrecht.

Al tijdens haar reguliere coschap Gynaecologie in het St. Antonius ziekenhuis in Nieuwegein ontdekte zij haar passie voor dit specialisme. Haar keuze-coschap en semi-arts stage volgde zij bij de afdeling Verloskunde in het Wilhelmina Kinder Ziekenhuis te Utrecht. Ook deed zij hier haar wetenschappelijke stage waaruit de publicatie "Ultrasonographic prediction of birth weight discordance in twin pregnancies" voortkwam.

Eind 2012 behaalde zij haar artsenexamen waarna zij als arts-assistent Gynaecologie in het UMC Utrecht heeft gewerkt. Een jaar later kreeg zij de kans om promotieonderzoek te doen onder leiding van prof. dr. Huub van der Vaart en dr. Mariëlla Withagen, wat heeft geleid tot dit proefschrift. Tijdens haar promotietraject heeft zij tevens de PEOPLE studie van het NVOG consortium grotendeels opgezet, een gerandomiseerde multicenter studie die vrouwen met een verzakking loot tussen een pessarium of een operatie. Hier zullen de komende jaren nog vele publicaties uit voortvloeien. Van 1 mei 2018 tot 1 oktober 2018 werkte zij als arts-assistent Gynaecologie in het St. Antonius ziekenhuis al waar zij op 1 oktober 2018 gestart is met de opleiding tot gynaecoloog (opleider dr. G.C.M. Graziosi).

Kim Verheijen – van de Waarsenburg is getrouwd met Bas Verheijen. Ze zijn de trotse ouders van hun dochter Lot (1,5 jaar) en wonen in Utrecht.