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Chapter 8

Evaluation of the Female Pelvic Floor in Pelvic Organ Prolapse using 3.0T Diffusion Tensor Imaging and Fibre Tractography.

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Chapter 8

Abstract

Purpose: To prospectively explore the clinical application of diffusion tensor imaging (DTI) and fibre-tractography in the evaluation of the female pelvic floor.

Material and Methods: Thirty women were consecutively included: 10 patients with pelvic organ prolapse, 10 patients with pelvic floor symptoms and 10 asymptomatic women. 2D SE-EPI sequence of the pelvic floor was performed. Offline fibre-tractography of principal pelvic structures was performed and two observers evaluated the tracking results. From agreed tracking results DTI parameters (eigen values $\lambda_1, \lambda_2$ and $\lambda_3$), mean diffusivity (MD), fractional anisotropy (FA) were calculated. Inter-rater agreement for quality assessment was evaluated using weighted kappa statistics ($\kappa$). Mean MD and FA values were compared among the three groups using variance analysis (ANOVA). Inter-rater reliability of DTI parameters was interpreted using intra-class correlation coefficient (ICC) statistics.

Results: For qualitative analyses, substantial inter-rater agreement was found ($\kappa=0.71\, (95\% CI0.63-0.78)$). Four anatomical structures (anal sphincter complex (AS), perineal body (PB), puboperineal muscle (PPM) and internal obturator muscle (IOM)) could be reliably identified. Substantial inter-rater agreement was found for MD and FA values (ICC 0.60-0.91). No significant differences between groups of MD and FA were observed for AS, PB and PPM. Significant difference in mean FA was found in the IOM-muscle between the prolapse group and nulliparous women (0.27±0.05) and (0.23±0.03) [p=0.013].

Conclusion: Currently, DTI with fibre-tractography permits three dimensional visualization of in-vivo pelvic floor anatomy, with reliable identification of part but not all of the clinically relevant anatomical structures. Overall no significant differences in DTI parameters were found between groups.
Introduction
Pelvic floor dysfunction entails a variety of conditions, including pelvic organ prolapse (POP), faecal and urinary incontinence. Certain basic risk factors have been considered, which among others include (complicated) vaginal delivery, normal aging and obesity [1,2]. Although its precise pathophysiology is currently only partly understood, defects in the levator ani muscle are known to be highly associated with the presence of pelvic floor prolapse [3]. Therefore, visualization of the pelvic floor musculature, identification of muscle injuries and in particular evaluation of muscle structure and function, would complement generally used clinical tools, and might reveal etiologic factors for pelvic organ prolapse.

Both static and dynamic Magnetic Resonance Imaging (MRI) enable a comprehensive interpretation of the complex pelvic floor anatomy and are increasingly proposed as a complementary diagnostic tool in pelvic floor dysfunction [4-7]. In the search for an improved three-dimensional (3D) understanding of anatomical relationships in the pelvic floor and changes in muscle integrity which might correlate with pelvic floor dysfunction, recently the use of diffusion tensor imaging (DTI) with fibre-tractography was proposed for the visualization of the normal female pelvic floor [8].

Recent studies have reported DTI as a useful method for detecting alterations in tissue organization of injured striated skeletal muscles as compared to normal muscles [9,10], which can be expressed in basic DTI parameters (i.e. eigen values, mean diffusivity (MD), and fractional anisotropy (FA)). The enhanced 3D visualization with DTI and fibre-tractography might have potential to visualize abnormal pelvic floor support in patients with pelvic floor prolapse, but has not been studied yet.

The purpose of this study was to examine the clinical application of diffusion tensor imaging (DTI) and fibre-tractography of the pelvic floor support, by prospectively evaluating and comparing the fibre-tract outcomes and basic DTI parameters of women with pelvic organ prolapse to women with pelvic floor symptoms, but without pelvic organ prolapse and to asymptomatic nulliparous women. In addition, the degree of inter-rater reliability was determined.

Material and Methods
Subjects
For this prospective cross-sectional study, institutional review board approval was obtained and all participants gave written informed consent. Three different groups of subjects were included, in total 30 female subjects were enrolled in this
study. Based on a published feasibility study [8], we anticipated the detection of (micro) structural differences between the symptomatic and asymptomatic pelvic support at already a small sample size.

The first group consisted of women with pelvic floor symptoms and had at least a stage 2 pelvic organ prolapse as staged with Pelvic Organ Prolapse Quantification (POPQ) staging criteria, according to the recommendations of the International Continence Society (ICS)[11]. The second group, which was age-matched with the prolapse group, consisted of women who had pelvic floor symptoms but without clinical relevant pelvic organ prolapse. The third group consisted of nulliparous women, without pelvic floor symptoms. All women were recruited in an outpatient clinic of a tertiary referral centre and underwent a pelvic examination during which the presence of a prolapse was assessed and staged [11]. Pelvic symptoms were measured in all subjects using a disease-specific symptom questionnaire, which was based on the Urogenital Distress Inventory (UDI), Incontinence Impact Questionnaires (IIQ) and Defecation Distress Inventory (DDI) items [12,13]. Exclusion criteria for all groups included previous pelvic floor surgery and general contraindications to undergo MRI (e.g. pacemakers, claustrophobia and pregnancy).

Image Acquisition

Each woman underwent MRI in the supine position with the legs parallel, slightly flexed using a 3.0T MR-scanner (Intera, Philips Healthcare, Best, the Netherlands) with a 16-channel phased-array surface coil (SENSE-XL-Torso, Philips Healthcare) for signal reception. No intravenous contrast medium was administered. Subjects were asked to empty the bladder 1 hour prior to the examination. MR imaging comprised the acquisition of a multishot Turbo Spin Echo (TSE) T1-weighted sequence (TR/TE: 600/10 ms, field-of-view (FOV): 200x200 mm², slice thickness: 5 mm, slices: 20 , echo train length: 7), multishot TSE T2-weighted sequence (TR/TE: 3021/80 ms, FOV: 300x300 mm², slice thickness: 4 mm, slices: 31 , slice gap 0.4mm, echo train length: 16) in axial, coronal and sagittal planes for anatomical reference and dual-echo gradient echo imaging to derive a B0-field inhomogeneity map (TR/TE1/TE2=12/4.6/9.6 ms, FOV: 200x200 mm², acquisition matrix: 80x80, pixel size: 2.5x2.5 mm², slice thickness: 5 mm, number of signal averages (NSA)=2). Axial two-dimensional (2D) diffusion weighted images were acquired with a diffusion-weighted Spin-Echo Echo-Planar Imaging (SE-EPI) pulse sequence using the following MRI parameters; TR/TE: 3750/40 ms, FOV: 200x200 mm², acquisition matrix: 80x80,
DTI in pelvic organ prolapse

pixel size: 2.5x2.5 mm², slice thickness: 5 mm, slices: 20, 32 diffusion weighted directions, NSA: 2, b= 400 s/mm², Spectral Adiabatic Inversion Recovery (SPAIR) for fat suppression). The total acquisition time for the DTI sequence was less than 4 minutes. The axial DTI sequence was non-angularly positioned and care was taken in order to place the anal sphincter complex and coccygeal bone within the FOV.

Post processing and Visualization
DTI data was processed using a custom build toolbox in Mathematica 8.0 [14]. Initially the data was filtered using a rician noise suppression algorithm [15], after which the diffusion-weighted data was registered to the non-weighted images using an affine transformation and corresponding b-matrix rotation [16]. Subsequently the diffusion tensor was calculated and corrected for field inhomogeneity induced deformations. The per-voxel absolute vector direction was colour coded according to standardized FA map colour coding: red indicating the left-right direction, blue the superior-inferior direction and green the antero-posterior direction. Offline fibre tracking was performed independently by two observers (FZ,MP), blinded to subject symptomatology and to prolapse status, using DTI software (DTITool, Biomedical Image Analysis group, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands (http://bmia.bmt.tue.nl/software/dtitool ) ). Observer 1 (FZ, a fourth-year resident in radiology with an additional 3-year experience as a teaching assistant at the department of Anatomy and Embryology, Academic Medical Centre, Amsterdam the Netherlands) and observer 2 (MP, medical doctor and third-year PhD student), were experienced with the complex pelvic floor anatomy and subsequent 3D DTI tractography [8].

In the acquired FA-maps and/or T1-weighted sequence, multiplanar, both manually drawn and positioned user-defined regions-of-interest (ROIs) were used to construct 3D fibre tracts of each of the predefined anatomical structures in the pelvic floor and wall (levator ani muscle (i.e. pubovisceral -, puborectal - and iliococcygeus muscle [17]), anal - and urethral sphincter complex, perineal body and internal obturator muscle) and the pelvic floor superficial layer (superficial transverse perineal -, bulbospongious muscle (also called bulbocavernosus muscle) and ischiocavernosus muscles). As the pubovisceral muscle consists of different subdivisions (with insertions on the level of the vagina (pubovaginal muscle), perineal body (puboperineal muscle) and the anal sphincter complex (punoanal muscle), respectively [17]), observers
were instructed to check whether these subdivisions could be separately visualized. Applied tracking parameters were standardized: minimal/maximal fibre length 10-100 mm; minimum/maximum FA thresholds 0.10-0.50; angle threshold <10-15°; integration step length 0.1 voxel. In case of a paired anatomical structure, the left and right muscle were tracked separately. The two symmetrical parts of both the bulbospongiousus – and puboperineal muscle were considered as an unpaired structure for analyses purposes as these structures were difficult to isolate as a paired structure.

**Qualitative data analysis**
The resultant 3D representation of each isolated anatomical structure was independently rated by both observers using a four-point scale (*good*=high-quality representation of the expected anatomical appearance within expected boundaries; *sufficient*=adequate representation of the expected anatomical appearance, based on fibre orientation, shape and location, but presence of focal tracking distortions and non-tracking; *insufficient*=non-satisfactory visualization with presence of only few fibre tracks and/or deviant fibre orientation, *not found*=not present). Subsequently, rates were categorized into satisfactory (i.e. *good* and *sufficient*) and non-satisfactory ( *insufficient* and *not found*). By using a consensus based method, individual 3D fibre trajectories were qualitatively evaluated and ROI’s were adjusted if necessary, resulting in a consensus based dataset.

Towards a better understanding of qualitative and quantitative fibre-tractography outcomes, the *pubovisceral muscle* was bilaterally evaluated on multiplanar 2D TSE T2w images using a grading system which was previously reported to assess (birth-associated) muscle abnormalities [18]. A four-point Likert scale was used to qualitatively assess the pubovisceral musculature (0=no visible defects; 1=less than half of muscle bulk lost; 2=more than half of muscle bulk lost; 3=complete muscle bulk lost) [18].

**DTI parameters**
For quantitative analysis, mean values (±SD) of the three eigen-values (λ1, λ2 and λ3) were calculated for multiple points per fibre along the resultant fibre tracts. Mean diffusivity (MD) and fractional anisotropy (FA) were defined as (D=Diffusion tensor):

\[
MD = \frac{1}{3} \text{Trace}(\mathbf{D}) = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} = \langle \lambda \rangle
\]  

[1]
DTI in pelvic organ prolapse

\[
FA = \sqrt{\frac{3}{2} \left( \lambda_1 - \langle \lambda \rangle \right)^2 + \left( \lambda_2 - \langle \lambda \rangle \right)^2 + \left( \lambda_3 - \langle \lambda \rangle \right)^2} \sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}
\]

[2]

Statistical Analysis

Interobserver agreement analysis was performed for each isolated anatomical structure. For the overall qualitative data (i.e. four-point scale quality assessment of 3D representation), inter-observer agreement was determined using quadratic weighted kappa statistics. Kappa statistics with 95% confidence intervals were calculated. For qualitative sub analyses, prevalence- and bias-adjusted kappa(κ) statistics (PABAK) were used.

DTI parameters (i.e. mean MD and FA values) were recorded and compared amongst the three groups if an isolated muscle was rated satisfactory (i.e. good or sufficient) in at least seven subjects per group. This cut-off was chosen to avoid statistical significant differences between groups based on substantial differences in group size. Per-muscle means for MD and FA values were compared among the three groups using a one-way analysis of variance (ANOVA). In case of significant difference (P< 0.05 ) a post-hoc student’s T-test with Bonferroni correction (P<0.016) was performed to study the significance among sets of two groups. Inter-rater agreement for the per-muscle quantitative data amongst the two independent observers was assessed using intra-class correlation analysis (ICC).

Results

From January 2010 to December 2010, thirty women were included in this study. One subject was not willing to complete the MRI examination after a technical interruption of the acquisition procedure. In one subject image quality was poor and therefore fibre-tractography was impossible, leaving 28 subjects for analyses. Baseline characteristics of the cohorts are shown in Table 1.

Qualitative analysis and inter-rater agreement

DTI with fibre-tractography was feasible in the 28 datasets, consequently resulting in 28 unpaired - and 56 paired anatomical structures. Fibre-tractography resulted in a satisfactory anatomical representation of the pubovisceral muscle in 34% (19/56); puborectal muscle in 13% (7/56); superficial transverse perineal muscle in 27% (15/56); ischiocavernosus muscle in 54% (30/56); bulbospongiosus muscle in 43% (12/28) and urethral sphincter
Figure 1. (a) Axial T2-weighted image of the pelvic area (ischial tuberosity (IT), pubic symphysis (PS)) of a 31-year old nulliparous woman, with projected three dimensional (3D) fiber tractography. Fiber tracts representing the anal sphincter complex (AS), perineal body (PB), transverse perineal muscle (TP) and puboperineal muscle (PPM). Vector directions are color coded: red indicating the left-right axis, blue the superior-inferior axis and green the antero-posterior axis. (b) An area of compact horizontally orientated fiber-tracts (red) is demonstrated on the level of the perineal body (PB), when fiber tracts are viewed from left anterolaterally. (c) In the same patient, the vector direction on the level of the anal sphincter complex (AS) reflects a predominating left-right (red) orientation at the anterior and posterior border and combined antero-posterior and superior-inferior (blue) orientation at the lateral border, indicating circular orientation from right lateral (c) and left anterolateral view (d).

complex in 29% (8/28) of the datasets, respectively. No perceptible differences in tractability or non-tractability were found in per-group distributions. The iliococcygeus muscle was rated non-satisfactory in all datasets (56/56). The following anatomical structures were identified in most of the DTI datasets: perineal body 100% (28/28), anal sphincter complex 93% (26/28) and internal obturator muscle 100% (56/56) (Figure 1). Despite the overall non-satisfactory visualization of the global appearance of the pubovisceral muscle in the data sets (37/56), analyses of its subdivisions resulted in a satisfactory visualization of the puboperineal muscle in 23 of the 28 subjects (Figure 2). Both the pubovaginal and puboanal subdivision could not or only insufficiently be tracked.
DTI in pelvic organ prolapse

Substantial overall inter-rater agreement was found for the independent qualitative scores. The overall weighted kappa for all muscle assessments was 0.71 (95% CI 0.63–0.78). Qualitative inter-observer agreement for the anatomical structures which met the criteria for quantification was also substantial (PABAK=0.76).

Table 1. Baseline characteristics of the study groups

<table>
<thead>
<tr>
<th></th>
<th>Nullipara</th>
<th>Age matched controls</th>
<th>Prolapse Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects per study group</td>
<td>9</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Age</td>
<td>27.7 (3.9)</td>
<td>52.7 (7.4)</td>
<td>58.0 (9.7)</td>
</tr>
<tr>
<td>BMI</td>
<td>21.5 (20.5-26.3)</td>
<td>22.2 (19.9-31.4)</td>
<td>24.3 (22.2-35.1)</td>
</tr>
<tr>
<td>Parity</td>
<td>0 (0-0)</td>
<td>2 (0-3)</td>
<td>2 (1-3)</td>
</tr>
<tr>
<td>POP-Q stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior compartment</td>
<td>0 (0-0)</td>
<td>0.5 (0-1)</td>
<td>2 (0-3)</td>
</tr>
<tr>
<td>Middle compartment</td>
<td>0 (0-0)</td>
<td>0 (0-1)</td>
<td>0.5 (0-2)</td>
</tr>
<tr>
<td>Posterior compartment</td>
<td>0 (0-0)</td>
<td>1 (0-2)</td>
<td>1 (0-2)</td>
</tr>
<tr>
<td>PVM injury</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>0 (0-0)</td>
<td>0 (0-2)</td>
<td>0 (0-2)</td>
</tr>
<tr>
<td>Left</td>
<td>0 (0-0)</td>
<td>0 (0-1)</td>
<td>1 (0-2)</td>
</tr>
</tbody>
</table>

Characteristics are expressed as median (range); BMI, body mass index; POP-Q, pelvic organ prolapse quantification; PVM, pubovisceral muscle

DTI parameters and inter-rater agreement

No statistical difference was detected in the mean values of MD between asymptomatic nulliparous women, age-matched controls and the prolaps group for the perineal body, anal sphincter complex, internal obturator muscle and the puboperineal muscle (Table 2). Mean MD values between groups, ranged from 1.35±0.09×10–6 mm²/s to 1.52±0.11×10–6 mm²/s and FA values between 0.22±0.03 to 0.27 ±0.04. Analysis of variance demonstrated no significant differences in mean diffusivity between the three groups for the perineal body, anal sphincter complex and the puboperineal muscle (Table 2). Mean FA value for the left obturator muscle was significantly lower in asymptomatic nulliparous women when compared to the prolaps group (mean FA 0.22±0.03 and 0.27±0.05 (p<0.05), respectively) which consequently resulted in a significant difference between these groups for the combined obturator DTI measures (Table 2).
Chapter 8

Table 2. Mean DTI values ± SD were compared between nulliparous, control, and prolapse group. DTI measures for the internal obturator muscle were combined.

<table>
<thead>
<tr>
<th></th>
<th>λ1 *</th>
<th>λ2 *</th>
<th>λ3 *</th>
<th>FA</th>
<th>p-value *</th>
<th>MD *</th>
<th>p-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Perineal body</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nullipara</td>
<td>1.79±0.18</td>
<td>1.33±0.16</td>
<td>1.00±0.14</td>
<td>0.25±0.06</td>
<td>.203</td>
<td>1.39±0.11</td>
<td>.877</td>
</tr>
<tr>
<td>Controls</td>
<td>1.67±0.18</td>
<td>1.29±0.16</td>
<td>1.03±0.16</td>
<td>0.24±0.05</td>
<td></td>
<td>1.36±0.14</td>
<td></td>
</tr>
<tr>
<td>Prolapse</td>
<td>1.90±0.44</td>
<td>1.50±0.38</td>
<td>1.18±0.26</td>
<td>0.24±0.06</td>
<td></td>
<td>1.51±0.36</td>
<td></td>
</tr>
<tr>
<td><strong>Anal sphincter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nullipara</td>
<td>1.76±0.13</td>
<td>1.37±0.11</td>
<td>1.08±0.13</td>
<td>0.25±0.04</td>
<td>.881</td>
<td>1.38±0.10</td>
<td>.340</td>
</tr>
<tr>
<td>Controls</td>
<td>1.76±0.30</td>
<td>1.40±0.20</td>
<td>1.09±0.22</td>
<td>0.25±0.04</td>
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<td>1.42±0.28</td>
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<tr>
<td>Prolapse</td>
<td>1.79±0.18</td>
<td>1.36±0.11</td>
<td>1.09±0.12</td>
<td>0.22±0.03</td>
<td></td>
<td>1.39±0.12</td>
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</tr>
<tr>
<td><strong>Internal obturator muscle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nullipara</td>
<td>1.80±0.16</td>
<td>1.38±0.13</td>
<td>1.12±0.10</td>
<td>0.23±0.03</td>
<td>.013</td>
<td>1.43±0.13</td>
<td>.870</td>
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<tr>
<td>Controls</td>
<td>1.84±0.18</td>
<td>1.39±0.17</td>
<td>1.09±0.16</td>
<td>0.25±0.04</td>
<td></td>
<td>1.44±0.18</td>
<td></td>
</tr>
<tr>
<td>Prolapse</td>
<td>1.91±0.27</td>
<td>1.42±0.17</td>
<td>1.08±0.15</td>
<td>0.27±0.05</td>
<td></td>
<td>1.46±0.15</td>
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<tr>
<td><strong>Puboperineal muscle</strong></td>
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<td></td>
</tr>
<tr>
<td>Nullipara</td>
<td>1.71±0.10</td>
<td>1.31±0.10</td>
<td>1.00±0.09</td>
<td>0.26±0.04</td>
<td>.127</td>
<td>1.35±0.09</td>
<td>.319</td>
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<tr>
<td>Controls</td>
<td>1.74±0.11</td>
<td>1.37±0.15</td>
<td>1.07±0.17</td>
<td>0.27±0.04</td>
<td></td>
<td>1.39±0.14</td>
<td></td>
</tr>
<tr>
<td>Prolapse</td>
<td>1.81±0.27</td>
<td>1.41±0.22</td>
<td>1.14±0.19</td>
<td>0.23±0.05</td>
<td></td>
<td>1.47±0.21</td>
<td></td>
</tr>
</tbody>
</table>

FA = Fractional Anisotropy; MD = Mean Diffusivity; * MD, λ1, λ2, λ3 are in units of [x 10⁻³ mm²/s]; † statistical differences between-groups using one way-ANOVA test; ‡ DTI measures of left- and right side were combined.

For the perineal body, anal sphincter complex, internal obturator muscle and the puboperineal muscle, the inter-rater agreement and 95% confidence interval (CI) for mean diffusivity and FA are summarized in Table 3. The intra-class correlation coefficient ranged between 0.60 and 0.91, which was interpreted as a substantial inter-rater agreement. Highest correlation between the two observers was found for the perineal body. The pubovisceral muscle status was normal for asymptomatic nulliparous women. Minor muscle injury (accumulated bilateral score 1-3, [18]) was observed for the age-matched control group. Both minor and major muscle injuries (accumulated bilateral score 4-6, [18]) were observed in the prolapse group. Median unilateral scores are expressed in Table 1.
DTI in pelvic organ prolapse

Table 3. Inter-rater agreement for Mean Diffusivity (MD) and Fractional Anisotropy (FA). Intraclass correlation coefficient (ICC) and 95% confidence interval (CI) are shown.

<table>
<thead>
<tr>
<th></th>
<th>ICC</th>
<th>95% CI</th>
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<tbody>
<tr>
<td><strong>Perineal body</strong></td>
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<td></td>
</tr>
<tr>
<td>FA</td>
<td>0.82</td>
<td>0.65 - 0.91</td>
</tr>
<tr>
<td>MD</td>
<td>0.91</td>
<td>0.81 - 0.96</td>
</tr>
<tr>
<td><strong>Anal sphincter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>0.76</td>
<td>0.55 - 0.88</td>
</tr>
<tr>
<td>MD</td>
<td>0.60</td>
<td>0.30 - 0.79</td>
</tr>
<tr>
<td><strong>Internal obturator muscle (R)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>0.76</td>
<td>0.55 - 0.88</td>
</tr>
<tr>
<td>MD</td>
<td>0.69</td>
<td>0.43 - 0.84</td>
</tr>
<tr>
<td><strong>Internal obturator muscle (L)</strong></td>
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<td></td>
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<tr>
<td>FA</td>
<td>0.70</td>
<td>0.44 - 0.85</td>
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<td>MD</td>
<td>0.74</td>
<td>0.52 - 0.87</td>
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<td><strong>Puboperineal muscle</strong></td>
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<td></td>
</tr>
<tr>
<td>FA</td>
<td>0.71</td>
<td>0.44 - 0.86</td>
</tr>
<tr>
<td>MD</td>
<td>0.66</td>
<td>0.37 - 0.84</td>
</tr>
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</table>

**Discussion**

Diffusion tensor imaging (DTI) permits a three-dimensional visualization and quantification of the pelvic floor anatomy, with reliable high-quality tractability of the anal sphincter complex, perineal body, the puboperineal muscle - a subdivision of the pubovisceral muscle - and the internal obturator muscle. However, not all pelvic support structures as initially defined could be reliably tracked. Basic DTI parameters could be determined with substantial inter-observer agreement. No apparent statistical significant differences in mean diffusivity (MD) and fractional anisotropy (FA) were demonstrated in the pelvic floor support between women with symptoms and stage 2 pelvic organ prolapse, women with symptoms and no prolapse and asymptomatic nulliparous controls. DTI characterizes and is able to visualize the local (micro) structural organization within anisotropic tissues, such as brain white matter [19] and striated skeletal muscle [20]. Technical feasibility of 3.0 Tesla DTI with fibre-tractography as a method to visualize the normal female pelvic anatomy was recently reported [8]. In that study, early estimates of the range of mean MD and FA values $(1.30 \pm 0.08 \times 10^{-3} \text{mm}^2/\text{s}$ to $1.73 \pm 0.12 \times 10^{-3} \text{mm}^2/\text{s}$ and $0.23 \pm 0.02$ to $0.30 \pm 0.04$,
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respectively) of pelvic floor structures in five healthy nulliparous women were provided. Current derived DTI parameters are concordant with these results.

Figure 2. (a) Tractography of the pelvic floor in a 70-year-old women with pelvic organ prolapse, representing the anal sphincter complex (AS), perineal body (PB) and puboperineal muscle (PPM) with an right anterolateral view, (b) anterior – and (c) posterior view. Vector directions are color coded: red= left-right axis; blue=superior-inferior axis; green=antero-posterior axis. Fiber tracts originating from the pubic bone with postero-inferior orientation of the vector as representation of the puboperineal muscle (PPM), reflected as an overall blue and green color coded fiber tract. Both fiber tracts bend medially to insert the perineal body (PB).
The association between levator ani muscle impairment and the presence of pelvic floor prolapse has been recognized [3]. Since DTI has been reported to be valuable in the assessment of muscle fatigue and defects [9], there was particular interest for the tractability of the pubovisceral muscle and its subdivisions (i.e. pubovaginal-, puboperineal- and puboanal muscle [17]), puborectal - and iliococcygeus muscle in the present three study groups. It is known that the iliococcygeus muscle, the most posterior part of the levator ani muscle, is often poorly developed and consequently difficulties in fibre tracking were expected and were confirmed during analyses.

Based on the initial hypothesis, potential alterations of water diffusivity as measured in the levator ani musculature were expected to be found in the prolapse group and to lesser extent in the age-matched group for the pubovisceral muscle. The pubovisceral muscle could be reliably tracked in a third of the women and the non-tractability of the pubovisceral musculature was proportionally distributed over the three study groups. In an earlier published pilot study in normal volunteers the pubovisceral muscle anatomy was globally demonstrated, although the highest variability in mean MD values was found for this muscle (1.49 ± 0.47×10⁻³mm²/s) and was attributed to its multifaceted organization [8]. The relatively complex architecture of the pubovisceral muscle together with the relative large voxel size and subsequent partial volume effects with potentially bending of effective diffusion vectors, made fibre tracking of the overall pubovisceral muscle in this study rather challenging with current applied MRI techniques. Despite these restrictions, assessment of the pubovisceral muscle resulted in a reliable 3D representation matching the global appearance of the puboanal muscle anatomy, which appeared as robust fibre trajectories originating from the pubic bone and inserting to the perineal body. Yet, no significant differences were found in DTI measures among groups.

The perineal body, located between the vagina and anal sphincter complex, serves as an anchor point for the pelvic floor. Recently, the anatomy of perineal body has been studied extensively by the Pelvic Floor Research Group with the use of thin-slice 3.0T MRI, in an attempt to enhance our understanding of this centrally located anatomical structure [6]. In that study, 3D models were created based on conventional MRI acquisition methods in women without pelvic floor dysfunction, which allowed organizing the complex anatomy in a superficial, mid and deep part [6]. With the usage of a 5 mm slice thickness we were able to observe the large number of fibre tracts transversing and bending in this complex area. The perineal body anatomy or central perineal tendon [21] could be tracked.
in all subjects, demonstrating an area of compact horizontally orientated fibre trajectories located caudally in the midline between the urogenital area and anal sphincter complex. Given the increasing interest in the perineal body, detailed demonstration of this anchor point at DTI could be a potential advantage of this technique.

Due to the uniform left-right orientation of the mean diffusion tensor in this area, the perineal body could easily be identified on colored FA-maps in all subjects and therefore fibre-tracts were obtained with high inter-rater agreement (ICC > 0.80 for mean FA and MD values). This in contrast to the overall pubovisceral muscle, where muscle detection was much less apparent owing to its complex anatomy with closely aligned muscles each with their own distinctive fibre direction. By assessing the different pubovisceral components during the analysis, we were able to overcome multidirectional tracking difficulties to a certain extent.

Significantly higher mean FA values in the left internal obturator muscle were found for women in the prolapse group, if compared to nulliparous women. The principal function of the internal obturator muscle is to abduct the flexed thigh and although it forms the most important element of the pelvic wall, it is not regarded as a pelvic support constituent. Yet, whereas the levator ani musculature anatomically originates partially from the tendinous ridge of the internal obturator muscle fascia, one might hypothesise a possible relation between levator ani muscular defects and subsequent changes in the internal obturator muscle on a microstructural level. In our study most levator ani muscle defects [18] in the prolapse group were observed unilaterally at the left side. Nonetheless, these results are still difficult to interpret.

The DTI fibre tracking process is reported to be highly user-dependent whereas the placement of the seeding ROI and the tracking criteria are determined by the individual observer [22]. By the use of predefined tracking parameters which were applied by both observers, the fibre tract stopping criteria were standardized. Also, by the application of consensus-based reading, an attempt was made to reduce the user dependence of this method. For the anal sphincter complex, perineal body, puboperineal muscle and the internal obturator muscle, high intra-class coefficients were found which can be interpreted as a substantial inter-observer reliability of DTI measures.

This study has recognized limitations. At present, we were not able to compare DTI measures of all clinical relevant anatomical structures of the pelvic floor support among the three groups, owing to the limited fibre tractability of
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some anatomical structures. Among others this might be contributed to applied technical parameters and technical improvements may overcome these limitations. The current study was performed using a 3.0 Tesla system, 16 channel surface coil and optimized scanning parameters, but still relatively large voxel dimensions were acquired (5×2×2 = 20 mm³) when compared to the commonly small diameter pelvic muscles. By increasing the number of acquisitions for the different encoding directions, an improvement of signal-to-noise ratio (SNR) and fiber-tracking precision can be obtained [23]. However, this will result in prolonged SE-EPI acquisition time (>10 minutes) which is associated with a higher likelihood of motion artifacts. Yet, spatial resolution can be optimized using dedicated coils, permitting the visualization of smaller anatomical structures and to minimize spatial volume effects. The use of an endoanal coil will ultimately result in an improved visualization of the perineal body anatomy and anal sphincter complex, but it will distort the local anatomy while the small effective volume will preclude evaluation of the complete pelvic floor. The latter can be overcome by using a combined pelvic and endoanal coil. Endosonography is widely used in clinical practice and shown to be as accurate as MRI for evaluating anal sphincter lesions [4]. However, this does not allow simultaneous evaluation of the complete pelvic floor support as in MRI.

Based on the published feasibility study [8], we anticipated the detection of (micro) structural differences between the symptomatic and asymptomatic pelvic support at already a small sample size. Yet, our current findings might result from substantial per-subject variation in tissue characteristics.

In conclusion, DTI with fibre-tractography allows for in-vivo 3D visualization of part of the pelvic floor support, with reliable visualization of the anal sphincter complex, perineal body, puboperineal muscle and the internal obturator muscle in women with pelvic organ prolapse (POP) as well as symptomatic age matched controls and asymptomatic nulliparous women. No significant differences were found in extrapolated per-muscle DTI parameters of the pelvic support, amongst the groups. Mean MD and FA values were assessed with substantial consistency by the different observers and overall SDs for the parameters were small. These initial results of DTI and tractography of the pelvic floor support in pelvic organ prolapse are encouraging and could be helpful to provide new insights in pelvic floor abnormalities and expand our three-dimensional understanding of this complex area. Further technical developments can be expected to lead to increased visualization of those muscular structures not or only partly visualized in this series.
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References