



Clinical and MRI changes of *puborectalis* and *iliococcygeus* after a short period of intensive pelvic floor muscles training with or without instrumentation

A prospective randomized controlled trial

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Abstract

Purpose This study evaluates the impact of a 3-week period of intensive pelvic floor muscles training (PFMT), with or without instrumentation, on clinical and static magnetic resonance imaging (MRI) changes of *puborectalis* (PR) and *iliococcygeus* (IL) muscles.

Methods 24 healthy young women were enrolled in the study and 17 achieved the 9 sessions of 30 min training exercises and conducted all assessments. Participants were randomly assigned in two training groups: voluntary contractions combined with hypopressive exercises (HYPO) or biofeedback exercises combined with transvaginal electrical stimulations (ELEC). Clinical and T2-weighted MRI assessments were realized before and after training.

Results Modified Oxford Grading System (MOGS) scores for left PR and perineal body significantly increased in the two groups ($p = 0.039$, $p = 0.008$), but MOGS score for right PR significantly increased only in HYPO ($p = 0.020$). Muscle volumes of right and left IL significantly decreased ($p = 0.040$, $p = 0.045$) after training as well as signal intensities of right and left PR ($p = 0.040$, $p = 0.021$) and thickness of right and left IL at mid-vagina location ($p = 0.012$, $p = 0.011$).

Conclusions A short period of intensive PFMT induces clinical and morphological changes in PFMs at rest suggesting a decrease in IL volume and adipose content of PR. Although the results suggested that an intensive non-instrumented PFMT is as effective as an instrumented training, future controlled studies with greater sample sizes are needed to establish the relative and absolute effectiveness of each of the two interventions.

Keywords Anatomy · *Levator ani* · *Transversus abdominis* · Strengthening · Morphology · Hypopressive · Biofeedback · Electrical stimulation

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Abbreviations

BMI	Body mass index
CONSORT	Consolidated standards of reporting trials
DA	Diaphragmatic aspiration
DTI	Diffusion tensor imaging
ELEC	Biofeedback exercises and transvaginal electrical stimulations group
HYPO	Voluntary contractions and hypopressive exercises group
ICC	Intraclass correlation coefficient
IL	<i>Iliococcygeus</i>
LA	<i>Levator ani</i>
MOGS	Modified Oxford grading system
MPL	Mid-pubic line
MRI	Magnetic resonance imaging

OI	<i>Obturator internus</i>
PB	Perineal body
PC	<i>Pubococcygeus</i>
PCL	Pubococcygeal line
PF	Pelvic floor dysfunction
PFDI-20	Pelvic floor distress inventory
PFIQ-7	Pelvic floor impact questionnaire
PFM	Pelvic floor muscle
PFMT	Pelvic floor muscle training
PLP	Posterior levator plate
PR	<i>Puborectalis</i>
PV	<i>Pubovisceralis</i>
RM ANOVA	Repeated measures analysis of variance
RT	Resting tone
SD	Standard deviation
TUI	Translabial tomographic ultrasound imaging
UI	Urinary incontinence

Introduction

Pelvic floor muscles (PFMs) play an important role in the maintenance of urinary continence and training of these muscles in women helps to prevent involuntary loss of urine (Dumoulin et al. 2014, 2015). Even if involuntary loss of urine is often considered a disorder of post-partum and post-menopausal women, this disorder is not exclusive to older women, as research has demonstrated a high prevalence of urinary incontinence (UI) among young, nulliparous female athletes and non-athletes (Jácome et al. 2011; van Breda et al. 2015). Therefore, prevention through pelvic floor muscle training (PFMT) is just as important in groups of young nulliparous women as in parous women.

Nowadays, numerous PFMT protocols could be used to prevent involuntary loss of urine and it is necessary to study the best ways to educate nulliparous women, specifically in younger age groups. PFMT can be achieved with instrumentation, using vaginal/ anal probe electrode delivering biofeedback exercises or electrical stimulations, and weighted vaginal cones or other resistance devices. It can be also achieved without any instrumentation, using direct voluntary contractions, also known as Kegel's exercises (Harvey 2003), or indirect contractions with *transversus abdominis* muscle activation through hypopressive exercises (Caufriez 1997; Resende et al. 2012).

Previous studies on women with pelvic floor dysfunction (PFD) show that PFMT without the use of electrical stimulation (Bø et al. 1999) or biofeedback (Fitz et al. 2012) is more efficient than with it, but this is controversial (Castro et al. 2008; Ibrahim et al. 2015). The effectiveness of Kegel's exercises in nulliparous women without clinical dysfunction has been questioned in a single-blind prospective

randomized controlled trial (Thorp et al. 1994). Uncertainty about which of these strategies are most effective in training women is one of the key clinical questions which needs to be prioritized because training using instrumentation is more costly than without. Therefore, further studies are needed to assess PFMT strategies efficacy.

From a physiological point of view, we believe that it is relevant to study the impact of PFMT in young asymptomatic women with “non-pathological” *levator ani* (LA) muscle signals that are preserved from the consequences of parity or loco-regional surgery. However, to the best of our knowledge, no study has investigated clinical and morphological changes in LA muscle subdivisions before and after PFMT in young asymptomatic nulliparous women, even though a recent study reported a high prevalence of 20% for involuntary loss of urine in a group of 159 young presumably healthy women aged 18–30 years (van Breda et al. 2015).

The purpose of this prospective randomized controlled trial was to evaluate the impact of a 3-week period of intensive PFMT, with or without instrumentation based on biofeedback and electrical stimulation, on clinical and static MRI changes of *puborectalis* (PR) and *iliococcygeus* (IL) muscles.

Methods

Study participants and PFM exercises

A sample of 24 healthy young nulliparous women (age: 22.9 ± 1.6 years, weight: 61.5 ± 6.9 kg, height: 167.7 ± 7.9 cm, body mass index (BMI): 21.8 ± 1.5 kg m⁻²), was recruited through advertisement and snowball sampling at our physiotherapy department. Before clinical and MRI assessments, all participants were questioned about PFD including symptoms of urinary and fecal continence, using a validated french version (de Tayrac et al. 2007) of Pelvic Floor Distress Inventory (PFDI-20) and Pelvic Floor Impact Questionnaire (PFIQ-7) (Barber et al. 2005). Additionally, we asked them if they were aware of their PFM function and if they known or already practiced PFM exercises. To be included in the study, participant had to have no MRI incompatibility (intrauterine device, pregnancy) or have practiced PFM exercises within the last year, a BMI < 30 kg m⁻², an intact perineum, and to be a nulliparous European woman aged < 30 years. Exclusion criteria were: pelvic floor disorders (stress urinary or fecal incontinence, detrusor overactivity, voiding or defecation dysfunction, vaginal or rectal prolapse), diarrhea or chronic constipation, intensive sports practices, claustrophobia, neuroleptic or antidepressant medication, abortion.

Participants were randomly assigned to two different PFM training groups. A first group realized voluntary PFM contractions combined with hypopressive exercises (HYPO) and the second followed biofeedback exercises combined with PFM electrical stimulations (ELEC). Participants were matched according to age (± 1 year) and BMI (± 0.5 kg m⁻²). Characteristics of the participants of the two groups at baseline are presented in Table 1 and no significant differences were observed for age, height, weight, and BMI. PFDI-20 and PFIQ-7 subscores were not significantly different between the groups at baseline (Table 1). The enrollment period was approximately 10–11 months. Participants of the two groups followed a total of 270 min (9 sessions of 30 min, 3 sessions/week during 3 weeks) PFMT.

In HYPO group, hypopressive abdominal exercises were instructed to the participants by 2 physiotherapists (E.G. and C.L.) and performed individually under their supervision, respecting the basic sequence proposed by Caufriez (1997): (1) slow and deep breathing in; (2) complete breathing out; (3) diaphragmatic aspiration (3 series of 8–12 repetitions). Breathing in was achieved by opening up the ribs, maximum breathing out by retracting the belly and completely releasing the air contained in the lungs, and diaphragmatic aspiration by acting as if one wanted to “suck one’s belly under the ribs” and maintain this position in apnea for 20 s. A standing, quadrupedic, and lying posture with active movements were selected, and the sequence of postures was left to the physiotherapist’s discretion. After diaphragmatic

aspiration, a voluntary contraction of PFM was achieved and sustained throughout the active movements. A complete session lasted 30 min.

In ELEC group, a commercially available electrical stimulator (Myomed 632, Enraf Nonius, Delft, the Netherlands) was used to deliver transvaginal electrical stimulations (excitomotor, bidirectional, rectangular, symmetric current) via vaginal probes (Goode et al. 2003) and lasts 15 min (450 s on right PR and 450 s on left PR) and biofeedback training lasts 15 min (6 s muscle contraction and 12 s of rest). Frequency stimulation was set between 20 and 50 Hz. Biphasic pulses had duration of 1 ms. The current intensity was adjusted to the maximum level that could be tolerated comfortably, up to maximum 100 mA.

Written informed consent was obtained from all individual participants included in the study. No financial compensation was provided to participate to the study. Institutional review board approval is not required at our institution for MRI using standard pulse sequences. A Consolidated Standards of Reporting Trials (CONSORT) diagram showing enrollment, training allocation, and follow-up is presented in Fig. 1.

Clinical and MRI assessments

Clinical assessment was realized in gynecological position (with the thighs flexed to 90°) by a physiotherapist who has specialized in uro-gynecology; before and after PFMT. At the first assessment, all participants were instructed on how to correctly perform their PFM using vaginal palpation. The physiotherapist was blinded to group allocation.

PFM strength was measured through an arbitrary 0–12 points scale using a highly reliable (Isherwood and Rane 2000) mechanical perineometer (PFX2, Cardio Design, Castle Hill, New South Wales, Australia) with a vaginal probe (26–28 mm diameter, active surface: 55 mm long, overall: 108 mm long). The cranial movement of the vaginal probe during measurement of PFM contraction was observed. Any contraction for which a retroversion of the hip or a Valsalva maneuver was noticed were discounted. PFM strength was also assessed by bi-digital palpation (Bø and Sherburn 2005) with the two distal phalanges inside the introitus vagina using the Modified Oxford Grading System (MOGS). It was individually assessed for right and left parts of PR and perineal body (PB), and consists of a 0–5 points scale: 0 = no contraction, 1 = flicker, 2 = weak, 3 = moderate, 4 = good (with lift) and 5 = strong. Three consecutive maximal contractions sustained for 5 s were recorded for perineometer and digital assessments, with a 30-s interval between efforts and the best of the 3 was registered.

PFM resting tone (RT) was quantified by digital palpation of left and right PR muscles (Dietz and Shek 2008) on a 6-point scale with the following grades: 0 = muscle not

Table 1 General characteristics of subjects and PFDI-20/PFIQ-7 subscores at baseline for the two groups

	HYPO	ELEC	<i>p</i>
Age (years)	22.9 \pm 1.2	23.0 \pm 2.0	0.889
Height (cm)	168.5 \pm 7.6	166.9 \pm 8.6	0.685
Weight (kg)	60.2 \pm 6.3	62.9 \pm 7.8	0.448
BMI (kg m ⁻²)	21.0 \pm 1.6	22.5 \pm 1.2	0.05
PFDI-20			
UDI-6 (/100)	17 [4–23]	12.5 [2–23]	0.806
POPDI-6 (/100)	8 [0–12.5]	8 [0–17]	0.576
CRADI-8 (/100)	0 [0–9.25]	8 [0–17]	0.222
PFIQ-7			
UIQ-7 (/100)	0 [0–9.5]	10 [2.5–35.75]	0.092
POPIQ-7 (/100)	0 [0–0]	0 [0–5]	0.239
CRAIQ-7 (/100)	0 [0–7]	0 [0–5]	0.633

Values are mean \pm SD for age, height, weight, and BMI. Values are median[1st–3rd] quartiles for PFDI-20 and PFIQ-7 subscores

BMI, body mass index; PFDI-20, pelvic floor distress inventory; UDI-6, urinary distress inventory; POPDI-6, pelvic organ prolapse distress inventory; CRADI-8, colorectal-anal distress inventory; PFIQ-7, pelvic floor impact questionnaire; UIQ-7, urinary impact questionnaire; POPIQ-7, pelvic organ prolapse impact questionnaire; CRAIQ-7, colorectal-anal impact questionnaire

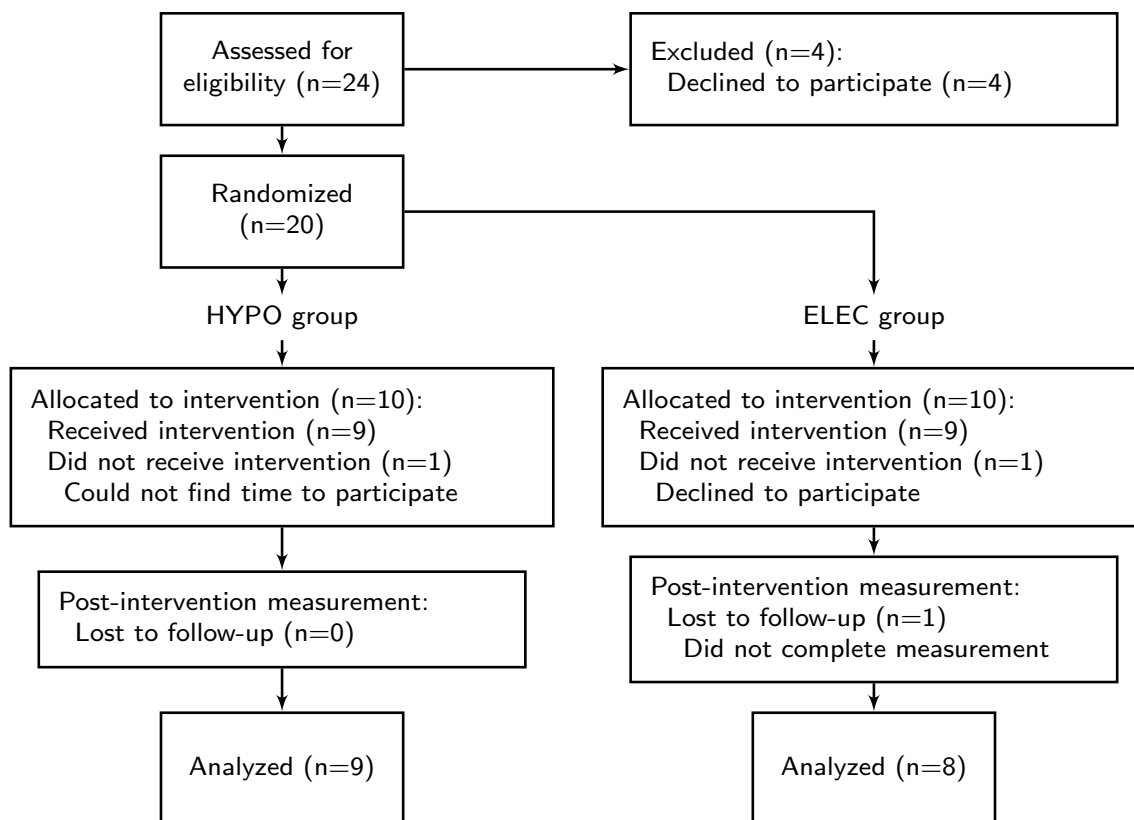


Fig. 1 Flowchart of participants' progress through the phases of the trial

palpable, 1 = muscle palpable but very flaccid, wide hiatus, minimal resistance to distension, 2 = hiatus wide but some resistance to distension, 3 = hiatus fairly narrow, fair resistance to palpation but easily distended, 4 = hiatus narrow, muscle can be distended, but high resistance to distension, no pain, and 5 = hiatus very narrow, no distension possible, 'woody' feel, possibly with pain: 'vaginismus'. PFM RT was computed as the sum of the grades of left and right parts of PR. Diaphragmatic aspiration (DA) (Caufriez 1997) was rated on a scale of 0–5–10 (0 = no movement, 5 = mobilization of the perineum or viscera, 10 = mobilization of the perineum and viscera). The steps of DA are as follows: slow diaphragmatic inspiration, followed by total expiration and, after glottal closure, a gradual contraction of the abdominal wall muscles, with superior displacement of the diaphragm cupola (Caufriez 1997; Resende et al. 2012; Santa Mina et al. 2015).

For MRI acquisition, each participant underwent an assessment performed in a supine position using a 1.5 T MRI scanner (Ingenia 1.5 T, Phillips, The Netherlands) and surface coils. Static T2-weighted turbo spin echo (TSE) techniques without fat saturation were used to image the sagittal, coronal, and axial planes during 20 min (spatial resolution 0.52×0.52 mm, repetition time (TR) 3745–4815 ms, echo time (TE) 90 ms, no gap, slices thickness 3 mm and

water phase shift minimum). Seventy images were obtained in sagittal plane, fifty in coronal and thirty-five in the PR paraxial plane. All participants were instructed by a medical imaging technologist to relax their PFM while breathing in and out normally. MRI assessments were performed before and after PFMT. All images were obtained at our nuclear magnetic resonance department. Different measurements were performed on images and are detailed in Table 2, Figs. 2 and 3. Mid-pubic line (MPL) (Singh et al. 2001), pubococcygeal line (PCL) (Yang et al. 1991), and posterior levator plate (PLP) (Hsu et al. 2006) were determined (see Fig. 2 and Table 2).

Clinical and MRI assessments were realized during the 7 days before the start of the training and the 7 days after the end of the training. Measurements required about 6 h for each participant and were performed by a radiologist who has specialized in pelvic floor MRI (L.M.) and two physiotherapists (E.G. and C.L.).

Sample size and statistical methods

A priori sample size was estimated from significant maximal voluntary contraction increase of PFMs after training obtained by Da Roza et al. (2012) on a sample of seven young, nulliparous sports students with UI. Considering the

Table 2 Measurements done on MRI

	Plane	Measurements (units)	Measurement sites	Sites checked	Figures
MPL–PCL angle	Midsagittal	Angle (°)	Between lower edge of symphysis pubis and last sacrococcygeal disc space	Through the bladder, urethra, vagina and rectum	2a
PLP–PCL angle	Midsagittal	Angle (°)	Between the point of intersection of levator plate- PCL and distal insertion	Through the bladder, urethra, vagina and rectum	2b
PR thickness	Para-axial (in PR axis, at the level of the inferior border of the pubic symphysis)	Maximum thickness (mm)	Mid-rectum and mid-vagina. At 9 and 3 o'clock the vagina	Sagittal	2c and 3a
PR height	Coronal	Maximum thickness (mm)	Mid-rectum	Sagittal	3a
IL thickness	Coronal	Maximum thickness (mm)	Mid-rectum and mid-vagina	Sagittal	2c and 3a
IL angle	Coronal	Angle (°)	Mid-rectum and mid-vagina. Angle formed by IL with horizontal plane of pelvis	Sagittal	2c and 3a
PR volume	Coronal	Contouring (cm ³)	All images where the muscle appear	Sagittal	3b
IL volume	Coronal	Contouring (cm ³)	All images where the muscle appear	Sagittal	3b
PR signal intensity	Axial	Relative signal PR/OI×100 (%)	At 9 and 3 o'clock the vagina and OI		3c
IL signal intensity	Coronal	Relative signal IL/OI×100 (%)	Through back of rectum		3d

Illustrations are given in Figs. 2 and 3

PR: *puborectalis*; IL: *iliococcygeus*; OI: *obturator internus*; MPL: mid-pubic line; PCL: pubococcygeal line; PLP: posterior levator plate

constitution of two groups and a probable sample loss of at least 20% in each group, the total sample size estimate was extended to 20.

All statistical procedures were performed with SigmaPlot software version 11.0 (Systat Software, San Jose, CA). The *p* value was considered significant when less than 0.05. Age, height, weight, and BMI differences at baseline between HYPO and ELEC groups were assessed with *t* tests. PFDI-20 and PFIQ-7 scores and subscores, and clinical results at baseline between HYPO and ELEC groups were compared using Mann–Whitney *U* tests and MRI results with *t* tests. Wilcoxon signed-ranked tests were performed to examine the effects of training protocols on clinical results in HYPO and ELEC groups. A two-way RM ANOVA (before–after, training groups) with Holm–Sidak method for pairwise multiple comparisons was performed to examine the effect of PFMT and training protocols on MRI results. All data are presented as means and standard deviations (SD) and were checked for normality (Shapiro–Wilk) and equal variance (Brown–Forsythe) tests.

For PR and IL volumes, both interobserver and intraobserver reliability were assessed using intraclass correlation coefficient (ICC(2,k): two-way random effects, absolute agreement, multiple raters ($k = 2$): E.G. and L.M.;

ICC(2,1): 2-way random effects, absolute agreement, single rater: E.G.) (Shrout and Fleiss 1979). Each observer's first measure recorded from each subject was used in the calculation of interobserver reliability. Intraobserver reliability was calculated using a second measurement recorded from each subject. ICCs were computed with R software (version 3.4.1) and irr package (version 0.84).

Results

Sample

Twenty women were randomized in the two groups and 17 were analyzed ($n = 9$ in HYPO and $n = 8$ in ELEC, see Fig. 1). The characterization of the sample groups revealed no statistically significant differences in all parameters. The groups are homogeneous and can be compared (Table 1). None of the participants reported any adverse effects from the PFMT programs. In HYPO group, all subjects were able to perform 30 min of hypopressive abdominal exercises and encountered no specific difficulties. During the first sessions, the more difficult was to maintain the 20-s apnea period.

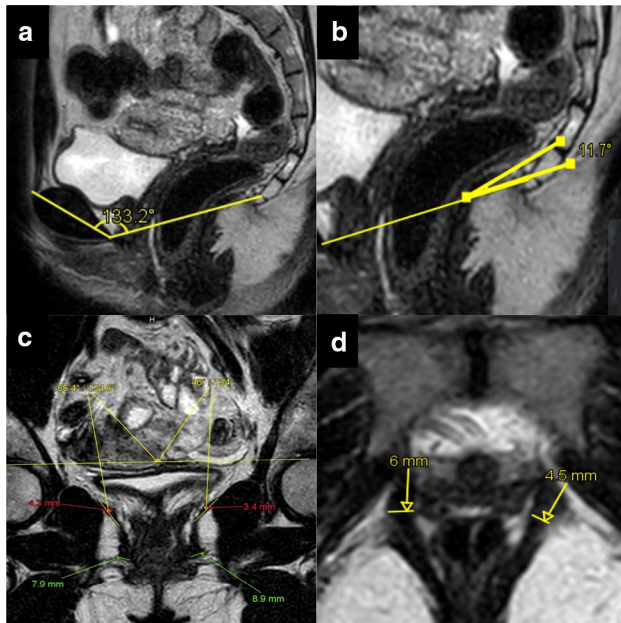


Fig. 2 Different IRM views showing measurements. **a** midsagittal plane: MPL–PCL angle; **b** midsagittal plane: PLP–PCL angle; **c** coronal plane to the mid vagina: PR thickness (green) and angle (yellow); **d** para-axial view: PR thickness. PR, *puborectalis*; IL, *iliococcygeus*; MPL, mid-pubic line; PCL, pubococcygeal line; PLP, posterior levator plate. (Color figure online)

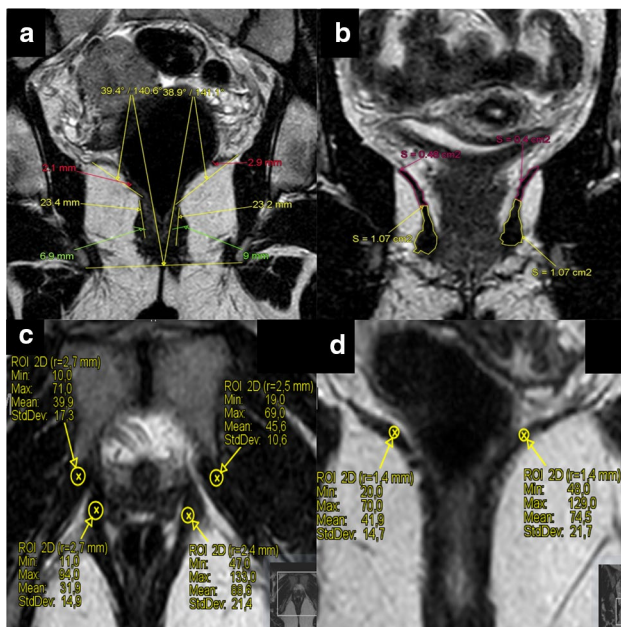


Fig. 3 Different IRM views showing measurements. **a** coronal plane to the mid rectum: PR thickness (green) and height (yellow), IL thickness (red) and angle (yellow); **b** coronal plane: PR (yellow) and IL (red) contouring; **c** axial plane: PR signal intensity; **d** coronal plane: IL signals intensity. Abbreviations are the same as in Fig. 2. (Color figure online)

Clinical results

No significant differences between groups were observed at baseline for all clinical results. MOGS scores for left PR and PB significantly increased in the two groups but MOGS score for right PR significantly increased only in HYPO group (Table 3). PFM strength and resting tone, and diaphragmatic aspiration were not modified.

MRI results

No significant differences between groups were observed at baseline for all MRI results. The ICC values were 0.70 (inter-) and 0.95 (intra-observer) for PR volume and 0.80 and 0.83 for IL volume, showing moderate to very good reliability results. Muscle volumes of right and left IL significantly decreased after training and signal intensities of right and left PR significantly decreased after training (Table 4). Muscle thickness of right and left IL at mid-vagina location significantly decreased after training (Table 4). MPL–PCL and PLP–PCL angles, PR volume, IL signal intensities, and PR and IL measurements done at mid-vagina, mid-rectum, and at 9 and 3 o'clock the vagina were not modified.

Discussion

LA muscle complex is one of the most important and complicated anatomical structure of the human body and as a result also one of the most poorly understood (Lammers et al. 2013). However, understanding the basic anatomy of the LA is essential when formulating a clinical opinion as injuries occur in 13–36% of women who have a vaginal delivery (Schwertner-Tiepelmann et al. 2012). Until now, no study explore the effects of a short period of intensive PFMT on the morphology of the pelvic muscles and related clinical scores in healthy nulliparous women, and the produced changes are uncertain. Our hypothesis was that the protocol based on biofeedback combined with vaginal stimulations would lead to less important morphological and clinical changes of PFM than voluntary contractions combined with hypopressive exercises. Results show the presence of clinical and morphological changes in different parts of LA muscle at rest after PFMT. However, changes were not significantly higher in the group that do not use instrumentation.

Historically, MRI studies have outlined the anatomy of PFM much more clearly than was possible with anatomical dissection studies (Raizada and Mittal 2008), and different subdivisions of the LA were identified (Kearney et al. 2004; Margulies et al. 2006). *Terminologia Anatomica* (Federative Committee on Anatomical Terminology 1998), identifies three major subdivisions of LA: IL, PR, and *pubococcygeus* (PC). Over the years, these subdivisions have been

Table 3 Clinical results

Training groups	Before (B)		After (A)		B versus A	
	HYPO	ELEC	HYPO	ELEC	<i>p</i> (HYPO)	<i>p</i> (ELEC)
PFM strength	4.5 [4–7.25]	4 [2.5–6]	5 [3–5.5]	5.25 [3.625–7.750]	0.461	0.094
MOGS rPR	3 [1.5–3.5]	4 [2–4]	4 [3.75–4.25]	4 [3.125–4.875]	0.020	0.078
MOGS lPR	2.5 [1.5–3.5]	3 [2–3.375]	3.5 [3–4.25]	3.75 [3–4.5]	0.039	0.016
MOGS PB	3 [1–4]	4 [1.25–4.375]	4.5 [3.75–5]	4 [3.5–5]	0.008	0.047
PFM RT	6 [4.25–8]	7.5 [5.25–8.875]	7 [4.5–8]	7 [7–8]	0.945	0.688
DA	10 [5–10]	7.5 [5–10]	10 [5–10]	7.5 [5–10]	1.000	1.000

Values are median [1st–3rd] quartiles. Significant values are in bold

PFM, pelvic floor muscles; MOGS, modified Oxford grading system; rPR, right *puborectalis*; lPR, left *puborectalis*; PB, perineal body; RT, resting tone; DA, diaphragmatic aspiration

Table 4 MRI results

Training groups	Before (B)		After (A)		<i>p</i>	B versus A	
	HYPO	ELEC	HYPO	ELEC		Training	B–A
Angle							
MPL–PCL	124.5 ± 2.0	127.3 ± 2.1	124.5 ± 2.0	125.7 ± 2.1	0.484	0.269	0.276
PLP–PCL	3.9 ± 0.7	5.6 ± 0.7	4.1 ± 0.7	5.5 ± 0.7	0.103	0.959	0.717
Volume							
rPR	5.908 ± 0.5	6.987 ± 0.6	5.879 ± 0.5	6.626 ± 0.6	0.195	0.576	0.634
lPR	5.970 ± 0.6	6.882 ± 0.6	6.171 ± 0.6	6.311 ± 0.6	0.482	0.604	0.288
rIL	5.563 ± 0.4	5.625 ± 0.4	4.309 ± 0.4	4.509 ± 0.4	0.727	0.040	0.897
lIL	5.720 ± 0.4	5.529 ± 0.4	4.487 ± 0.4	4.578 ± 0.4	0.900	0.045	0.781
Signal intensity							
rPR	223.8 ± 14	228.4 ± 16	187.4 ± 14	194.2 ± 16	0.722	0.040	0.945
lPR	223.3 ± 13	221.4 ± 15	185.6 ± 13	176.5 ± 15	0.695	0.021	0.826
rIL	291.9 ± 36	262.5 ± 41	270.4 ± 36	328.4 ± 41	0.681	0.632	0.353
lIL	326.3 ± 33	242.2 ± 36	286.0 ± 36	279.4 ± 37	0.198	0.967	0.330
Mid-vagina							
rPR thickness	9.2 ± 0.7	9.4 ± 0.7	8.6 ± 0.7	9.0 ± 0.7	0.630	0.436	0.802
lPR thickness	8.7 ± 0.6	9.3 ± 0.7	8.4 ± 0.6	9.3 ± 0.7	0.636	0.742	0.762
rIL thickness	3.5 ± 0.3	4.0 ± 0.4	2.5 ± 0.3	2.9 ± 0.4	0.224	0.012	0.970
lIL thickness	3.8 ± 0.4	3.6 ± 0.4	2.7 ± 0.4	2.9 ± 0.4	0.955	0.011	0.398
rIL angle	55.0 ± 2.6	55.7 ± 2.8	54.3 ± 2.6	61.1 ± 2.8	0.292	0.191	0.098
lIL angle	58.1 ± 2.8	53.7 ± 2.9	55.1 ± 2.8	56.3 ± 2.9	0.674	0.896	0.081
Mid-rectum							
rPR thickness	8.7 ± 0.9	8.0 ± 1.0	8.7 ± 0.9	8.0 ± 1.0	0.562	0.973	0.973
lPR thickness	8.4 ± 0.8	8.3 ± 0.9	8.5 ± 0.8	8.0 ± 0.9	0.728	0.890	0.794
rPR height	16.8 ± 2.2	20.7 ± 2.4	18.2 ± 2.2	24.1 ± 2.4	0.075	0.254	0.636
lPR height	16.7 ± 2.1	20.5 ± 2.2	18.6 ± 2.1	23.6 ± 2.2	0.118	0.103	0.690
rIL thickness	3.6 ± 0.4	4.0 ± 0.4	3.2 ± 0.4	4.2 ± 0.4	0.091	0.666	0.358
lIL thickness	4.0 ± 0.4	4.2 ± 0.4	3.1 ± 0.4	4.3 ± 0.4	0.149	0.258	0.119
rIL angle	46.4 ± 2.7	44.5 ± 2.9	44.4 ± 2.7	41.3 ± 2.9	0.485	0.143	0.716
lIL angle	46.0 ± 2.7	43.5 ± 2.8	46.5 ± 2.7	39.6 ± 2.8	0.167	0.419	0.296
9 and 3 o'clock vagina							
rPR thickness	8.0 ± 0.9	7.8 ± 0.9	7.4 ± 0.9	8.0 ± 0.9	0.854	0.717	0.585
lPR thickness	8.2 ± 0.9	8.0 ± 1.0	8.1 ± 0.9	7.8 ± 1.0	0.848	0.820	0.971

Values are mean ± SD. Significant values are in bold. All angles are expressed in degrees, volumes in cm³, signal intensities in %, and distances in mm

given several names, making understanding even more difficult (Lammers et al. 2013). Among female, PC muscle, also called *pubovisceralis* (PV), is further divided into the *puboperinealis*, *pubovaginalis*, and *puboanalis* (Lawson 1974; Kearney et al. 2004). Even if different parts of PV and enclosing urogenital hiatus, could be observed with standard MRI techniques, they can not be distinguished at their origin of the pubic bone (Zijta et al. 2013). Only MRI studies with diffusion tensor imaging (DTI) and fiber tractography allowed to provide a three-dimensional overall appearance of muscular fibers of PV (Zijta et al. 2011; Rousset et al. 2012), and it was, therefore, decided not to study PV. From a methodological viewpoint, both MRI and translabial tomographic ultrasound imaging (TUI) are relevant tools to study the morphological changes in PFMs after training. However, morphological changes of the IL are difficult to detect using TUI (Yan et al. 2017). Today, we believe that MRI is the most relevant tool to observe morphological changes of the IL.

From a morphological viewpoint, PR is thicker than IL and forms a band around the urethra, vagina and rectum (Singh et al. 2002). Our results show that PR thickness values before PFMT were greater at mid-vagina level compared to other levels, with mean values between 8.7 ± 0.6 and 9.4 ± 0.7 mm, and IL thickness values were greater at mid-rectum level, with mean values between 3.6 ± 0.4 and 4.0 ± 0.4 mm. Singh et al. (2002) observed lower thickness values for PR (6.5 ± 2.0) and IL (2.9 ± 0.8), but their sample was composed of healthy nulliparous women aged between 23–42 years. Dumoulin et al. (2007) showed in women with stress urinary incontinence that LA surface area at rest after PFMT was significantly smaller than before training; however, during a voluntary contraction, LA surface was significantly higher than before training. Even in healthy women, our results are in agreement with this study since we observed a decreased volume and thickness of IL muscle at mid-vagina level, both for PFMT with and without instrumentation. A dynamic MRI study is, therefore, needed to complete our static results. Furthermore, PR muscle volume and thickness were unchanged but signal intensity of PR compared with that of OI decreased, both for PFMT with and without instrumentation. The signal intensity of healthy muscles, i.e., muscles showing no muscle edema, fatty infiltration, or mass lesion, is well lower than that of fat for T2-weighted images (May et al. 2000). Hence it can be argued that the decreased signal intensity of PR indicates a decrease in fatty composition of this muscle, in favor of an increase of muscle fibers. Since stress urinary incontinence, apart of ligamentous and fascial lesions, is associated with increased signal intensity (Kirschner-Hermanns et al. 1993), this observation could be of major importance in pathological conditions. From a clinical viewpoint, MOGS scores significantly increased after PFMT, indicating a global increase of PFM strength.

However, MOGS score for rPR in ELEC group was not significantly increased after PFMT compared to IPR. This observation may be explained by the number of participants with high MOGS scores (scores ≥ 4) in ELEC group for rPR before training (MOGS at 4: $n = 4$, MOGS at 5: $n = 1$) compared to only one participant with a MOGS score for IPR at 4. We believe that a 3-week period was too short to reach a significant difference in MOGS score for rPR since the baseline score was high for most participants of the ELEC group. Since a 3-week period of intensive PFMT, based on 90 min/week, was sufficient to increase muscle strength in the 2 training groups, we believe that, in a preventive purpose, it is not necessary to use an expensive and more invasive method than combined voluntary and hypopressive exercises (Bernardes et al. 2012).

PFM strength changes observed after PFMT and assessed with perineometer were not significant. Perineometer values represent a global measurement of vaginal closure force that also includes the “noise” caused by the rise in intra-abdominal pressure that often accompanies a LA contraction (Ashton-Miller et al. 2014). Even if we systematically checked the presence of a cranial movement of the vaginal probe during the measurement, we can exclude an increase in intra-abdominal pressure that could have masked the finding of a significant increase in the vaginal closure force after PFMT. The use of an instrumented speculum designed to minimize the effect of intra-abdominal pressure during measurement of PFM strength and developed by Ashton-Miller et al. (2014), may be a solution to overcome this limitation. Another explanation of these results could be that the global maximal vaginal closure force was not modified but that some muscles have increased their strength to the detriment of others. Our hypothesis is based on the following observations after PFMT: (1) the decreasing volume and thickness of IL at the level of mid-vagina, without any change in signal intensity; and (2) the decrease of signal intensity in PR. Therefore, IL force may have been reduced while PR force increased. If this is the case, PFMT could be effective in modifying the resulting forces of the different parts of LA since the lines of action of IL (PV) and PR muscles are quite different: the first one lifts the pelvic floor while the second closes it (Betschart et al. 2014). The absence of a change in signal intensity of IL must be balanced according to the training groups. Surprisingly, signal intensity tends to increase in ELEC group while it decrease in HYPO. This finding also support the use of non-instrumented PFMT.

The present study has several limitations. The first limitation is the small number of the participants; therefore, our results may be not representative of the general population. To reduce bias about group homogeneity, several inclusion criteria were met. The first inclusion criterion was that all participants were young and nulliparous women. According

to Slieker-ten Hove et al. (2009), voluntary muscle contraction decrease with age, but there is no relation with parity. DeLancey et al. (2003) found abnormalities in the LA muscle on MRI after vaginal delivery, that are not found in nulliparous. In women over age 60 years, the PFMs are significantly thinner both at rest and during contraction, compared to younger women (Bernstein 1997). The second criterion was that participants were European women to avoid ethnic variability. Indeed, functional and morphological differences in the urethral sphincter and support system of nulliparous black and white women were previously observed (Howard et al. 2000; Handa et al. 2008). Even if homogeneity between the groups at baseline was adequate (see Table 1), note that BMI values between the groups were almost significantly different ($p = 0.05$). We included subjects with $BMI < 30 \text{ kg m}^{-2}$. However, even if we observed a statistical significant difference for this parameter, this observation will be not relevant since BMI values observed in both groups are even far away from the cut-off value of 25 kg m^{-2} for overweight adult subjects. The second limitation is the very short intervention period of 3 weeks. The choice of this short period is in accordance with Belgian health-insurance mutuels that usually reimburse 3 weeks of urogynecological physiotherapy rehabilitation for the treatment of female UI. We acknowledge that a longer period could lead to different clinical and MRI observations. The third limitation is that PFMs were only assessed with static MRI and a dynamic MRI study is needed to complete our results (Cai et al. 2013; Dumoulin et al. 2007). Others limitations relate to MRI acquisition. Each participant underwent MRI adapted at supine position with a mid-full bladder repletion. In the supine position, PFMs do not undergo organ weight and gravity (Fielding et al. 1998). A mid-full bladder repletion condition is difficult to obtain because of the ability of each bladder. During image analysis, we observed different degree of repletion of the bladder, which could influence our results. Finally, signal measurements were difficult to do coronally in IL (very thin) and in some PR and IL frequently fasciculated with fat striations.

Conclusions

This study suggests that 270 min of PFMT in healthy nulliparous women produces clinical and morphological changes in PFMs at rest. Muscle volume and thickness of IL at mid-vagina location decreased after training. PR volume was not modified but its signal intensity decreased, indicating a decrease in fatty composition of this muscle, in favor of an increase of muscle fibers. Additionally, MOGS scores increased after training, indicating that participants improved voluntary force contraction of their PFMs. Static MRI appears to be a relevant tool to highlight the impact

of PFMT but is not sufficient to fully explain all clinical improvement and morphological changes. Further dynamic MRI studies and/or DTI-based tractography are needed to better understand the impact of training on the morphology of PFMs, and determine if these changes are sustainable and applicable to women with PFD.

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Author contributions FD and AFB conceived and designed research. FD, EG, CL and LM conducted experiments. FD, EG and CL analyzed data. FD, EG, CL, FB, AFB and LM wrote the manuscript. All authors read and approved the manuscript.

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