**The facilitatory effect of duloxetine combined with pelvic floor muscle training on the excitability of urethral sphincter motor neurons.**

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**Abstract:**

Introduction and Hypothesis:

Aim of this study was to investigate the excitability of sphincter motor neurons under the influence of pelvic floor muscle training (PFMT) and duloxetine. Due to their mechanisms of action there might be a synergistic effect of duloxetine and PFMT in regard to the facilitation of spinal reflexes controlling urethral sphincter contractions and hence continence.

Methods:

In 10 healthy female subjects clitoral electric stimulation (CLES) and transcranial magnetic stimulation (TCMS) were used to determine individual motor thresholds for external urethral sphincter (EUS) contractions before and after PFMT, duloxetine, and PFMT + duloxetine.

Results:

PFMT and duloxetine alone significantly decreased the motor thresholds for EUS contractions during CLES and TCMS. However, the combined treatment reduced the motor threshold for EUS contractions significantly stronger compared to PFMT or duloxetine alone.

Conclusions:

The results are suggestive for a synergistic facilitatory effect of PFMT and duloxetine on sphincter motor neuron activation.

**Summary:** Duloxetine combined with pelvic floor muscle training seems to have a significant synergistic effect decreasing urethral sphincter motor thresholds during transcranial magnetic and bulbocavernousus reflex stimulation.

**Introduction**

Stress urinary incontinence (SUI) – defined as the complaint of involuntary leakage on effort or exertion, or on sneezing or coughing [1] - is an often bothersome symptom that reduces the quality of life (QoL), including sexual health [1, 2]. SUI occurs when bladder pressure exceeds urethral closure pressure under conditions of increased abdominal pressure. The peak incidence of SUI occurs between 45 and 49 years of age and obesity, pregnancy, and vaginal childbirth are recognized risk factors [2]. The prevalence of SUI is about 50% among women with urinary incontinence (UI) [3]. UI in women is a common disorder and the median worldwide prevalence is indicated with 27.6% and is considered even higher in institutional settings [4].

Different factors can have an influence on the pathogenesis of SUI [5] and several theories exist regarding the underlying mechanism or dysfunction causing SUI, including urethral hypermobility and intrinsic sphincter deficiency [6, 7], the "backboard" or "hammock" concept [8] and the integral theory [9]. Accordingly, there are different treatment options correcting the assumed cause of SUI, including different urethral sling surgeries, colposuspension, urethral bulking agents, vaginal pessaries, and pelvic floor muscle training (PFMT).

Drug treatment for SUI played only a subordinate role until duloxetine, a combined serotonin and norepinephrine reuptake inhibitor, was introduced in the treatment of SUI a few years ago and showed promising results [10]. Its main mechanism of action in SUI treatment is to increase the tone of the external urethral sphincter by increased availability of the excitatory neurotransmitters serotonin and norepinephrine in the sacral spinal cord, where the sphincter motoneurons are located [11-13].

Although PFMT, a recommended first line therapy for SUI, and duloxetine, as a first drug of its class used in SUI, are shown to be effective conservative treatment options, little is known about a combined effect of both therapies.

Up to day there is only one randomized controlled clinical trial investigating the combined treatment of PFMT and duloxetine in women with SUI [14]. The results suggested an additive effect of a combined treatment. The aim of this study was now, based on previous experience and findings [11], to explore a possible working mechanism and reason for the potential benefit of the combined therapy, using neurophysiological and urodynamical measurements. Our hypothesis is that duloxetine in combination with PFMT has a synergistic effect on the excitability of pudendal motor neurons, more than duloxetine or PFMT alone.

**Subjects and Methods**

After approval of the local ethics committee (Kantonale Ethikkommission Zürich), a volunteer sample of healthy females was recruited. Inclusion criteria: healthy female, age 18 to 30 years. Exclusion criteria: urinary tract infection, pregnancy, previous child birth, any current health problem or medication, any past or current lower urinary tract disorder and any allergy to duloxetine. All subjects were informed in written and oral form and had to provide written informed consent prior to inclusion.

Urinary tract infection and pregnancy were excluded prior to the investigation, using urine dip stick tests.

Before starting the experiment, PFMT was explained to the subjects in detail and correct contraction was assessed by intravaginal manual control, requiring a maximal contraction around the finger and a slight inward lift, without straining and contraction of the abdominal muscles or lifting the whole pelvis due to contraction of the buttocks. The PFMT instruction, the exercises itself and all other measurements during the experiment were performed with subjects in supine position.

The experiment consisted of 4 measurements, during which the individual resting motor threshold for external urethral sphincter (EUS) contraction in response to clitoral electric stimulation (CLES) and transcranial magnetic stimulation (TCMS) was determined (Fig. 1).

Prior to measurement 1, a 8 Fr Microtip catheter (Unitip, Unisensor AG, Attikon, Switzerland) was inserted transurethraly and the bladder was filled by 100 ml. The urethral pressure transducer of the catheter was placed at the point of maximum urethral pressure and fixated properly with tape on the inside of the left thigh. Additionally, a vaginal probe (Periform intravaginal probe, Neen, Oldham, UK) with a length of 8 cm was placed. The position of both, the catheter and the vaginal probe was assessed prior to each measurement or PFMT using fluoroscopy (Fig. 2).

CLES was performed using a bipolar stimulator (AS100, ALEA Solutions GmbH, Zürich, Switzerland), which was connected to two surface disc electrodes beside the clitoris. The current was slowly increased until a contraction response could be observed from the pressure transducer at the EUS.

TCMS was performed using a liquid cooled magnetic coil (MC125, Dantec Medical A/S, Skovlunde, Denmark) connected to a magnetic stimulator (MagPro, Dantec Medical A/S, Skovlunde, Denmark) with a maximum magnetic field strength of 1.8 Tesla. During stimulation, the coil was positioned in midline over the cranial motor cortex and after determination of the area of best response (hot spot), the magnetic field strength was slowly increased until a contraction response could be observed from the pressure transducer at the EUS. CLES and TCMS were repeated alternately twice per measurement, to exclude a mutual effect and to assess reproducibility.

Between measurement 1 and 2, and 3 and 4, PFMT was performed for 10 minutes with a contraction frequency of 0.5 Hz under biofeedback monitoring (AutoMove AM800 by Danmeter A/S, Odense, Denmark) with 1 minute of training alternating with 1 minute of rest

(Fig. 1). All subjects were requested to perform maximum pelvic floor muscle contractions during the training, which was controlled via the biofeedback display.

Between measurement 2 and 3, subjects received 40 mg of duloxetine (Cymbalta®, Eli Lilly SA, Vernier/Genève, Switzerland) and had a 4 hour rest, during which the catheter and vaginal probe were removed. After measurement 2 and 4, uroflowmetry and measurement of post void residual volume was performed.

The mean of the two thresholds determined with CLES and TCMS during each measurement were compared between the individual measurements using the Wilcoxon signed ranks test in SPSS 14.0 (SPSS Inc. Headquarters, 233 S. Wacker Drive, 11th floor, Chicago, Illinois 60606). Due to multiple comparisons, α was corrected to 0.016 (Bonferroni method).

**Results**

Ten healthy female subjects (age: 24.5 ±2.9 years) could be included (Table 1). The experimental procedure was well tolerated and all subjects completed the study. Side effects were limited to nausea in two and tiredness in four subjects. None of the subjects indicated pain during catheterisation, magnetic stimulation or electrical clitoris stimulation.

Individual motor thresholds for EUS contraction could be obtained in response to CLES and TCMS during all 4 measurements in all subjects (Table 1).

*Effect of PFMT on the excitability of EUS neurons (measurement 1 vs. 2)*

After PFMT, the individual motor thresholds for EUS contractions during CLES and TCMS were significantly lower (Fig. 3, 4).

*Effect of duloxetine on the excitability of EUS neurons (measurement 1 vs. 3)*

4 hours after duloxetine intake, the individual motor thresholds for EUS contractions during CLES and TCMS were significantly lower (Fig. 3, 4).

*Combined effect of duloxetine and PFMT on the excitability of EUS neurons (measurement 2 vs. 4 and 3 vs. 4)*

After combined treatment with duloxetine and PFMT, the individual motor thresholds for EUS contractions during CLES and TCMS were even significantly lower compared to PFMT or duloxetine alone (Fig. 3, 4).

**Discussion**

According to our hypothesis, duloxetine and PFMT combined significantly reduced the motor thresholds for EUS contractions even more compared to duloxetine or PFMT alone.

PFMT in the treatment of SUI can presumably work via three different ways: a) conscious pelvic floor muscle pre-contraction during physical stress, b) strength training, or/and c) indirect training of the pelvic floor muscles via abdominal muscle training [15]. An optimal outcome of PFMT for SUI patients would be to reach the automatic (unconscious) co-contraction level, which is present in healthy continent subjects. How this result can be obtained best, whether with option a), b), c) or in combination is still unclear [15]. The evidence for the efficacy of indirect training via abdominal muscles (option c) is however poor. To date there is only one study comparing PFMT with PFMT + transversus abdominus training, finding no benefit of adding transversus abdominus training [16].

Regarding the facilitatory neuromodulative effects of muscle training, it could be demonstrated in several studies that even short-term muscle training increases the excitability in supraspinal and also in spinal centres [17, 18]. This is one of the first mechanisms together with adaptations in motor unit recruitment which is involved in the very early changes after

starting muscle training [18]. The supraspinal regions involved in EUS control and coordination of pelvic floor muscles including the EUS have been revealed in several imaging studies using PET and fMRI [19]. As our findings demonstrate, PFMT not only decreases the motor threshold during TCMS but also during CLES, which is in principle nothing else than bulbocavernosus reflex testing. This would possibly implicate that PFMT not only cause changes in supraspinal regions but also in the sacral spinal cord in regard of facilitating sacral viscero-somatic reflexes.

PFMT is yet the first line therapy for SUI, mixed urinary incontinence and sometimes even urgency urinary incontinence and based on the available data, it seems that PFMT is better than no treatment, placebo, or inactive control treatments [20]. Short term cure rates with PFMT are encouraging with rates of 44-70% ($\leq 2$ g of urine leak on pad test), although no consensus exists regarding a gold standard measure for cure [15].

It can take however up to 4 to 8 weeks to improve strength and/or timing of PFM contractions and as long as 5 months to show a clinical improvement in SUI for the patients [15, 21].

Therefore, the efficacy and long term results of PFMT, next to a skilled training education by a specialised physiotherapist, very much relies on the motivation and compliance of the patient. Compliance and motivation is even more important as PFMT should be continued indefinitely following a certain standardised regimen [22].

Although most studies on long term follow up are difficult to compare due to differences in training regiment, outcome measures and long term compliance with PFMT, the results of some studies show that in the majority of patients a beneficial effect of PFMT can be maintained and that the patients do not require surgery anymore. However, there is still a need for a large randomized placebo-controlled long-term study on the clinical effectiveness and quality of life, which is in addition sufficient for subgroup analysis [20].

Regarding the cost effectiveness, PFMT with a ratio of €0.03/incontinence episode (IE) avoided, is favourable over duloxetine with a ratio of €3.81/IE (the underlying calculations refer to the Netherlands. The results can not necessarily applied to other countries) [23].

Duloxetine is a first pharmacological therapy option for SUI. It can not completely cure SUI and 40 mg bid used for SUI treatment can cause several adverse events (most commonly: nausea, vomiting, constipation, headache, dry mouth, fatigue, dizziness and insomnia) appearing in 1-25% of patients receiving duloxetine in trials, which resulted in discontinuation of the drug in about 1 of 8 people [10]. Although it is approved in several european countries since August 2004 for the treatment of SUI, it is not approved by the United States Food and Drug Administration (FDA) due to safety concerns (www.fda.gov). Nevertheless, it could be shown in several randomised controlled trials, that duloxetine is superior to placebo and can significantly reduce incontinence and improve QoL [10]. It has a relatively balanced combined inhibitory effect on the synaptic reuptake of serotonin (5-Hydroxytryptamin, 5-HT) and norepinephrine (NE). Although α-adrenoceptors and 5-HT receptors can be found throughout the human body and with high density in several areas in the spinal cord, there is a specific high receptor density in the sacral ventral horns of the spinal cord, in the area of the Onuf nucleus, where the urethral sphincter motoneurons are located [13]. Thor and Katofiasc could demonstrate in their study with anaesthetized cats, that duloxetine was able to decrease bladder contractions and increase bladder capacity. This effect was even more pronounced in cat bladders, treated with acetic acid. Additionally, duloxetine showed an increase in muscle activity of the EUS [13]. Both effects, on bladder capacity and sphincter activity increased dose dependently. However, systemic administration of duloxetine did not result in inhibition or decrease of bladder contraction evoked by direct stimulation of the pelvic nerve, which suggests an effect of duloxetine rather in the central nervous system than peripheral. Further studies in cats revealed that the receptors 5-HT2 and α1 mediate the facilitation of pudendal nerve reflexes [24, 25]. A study in humans emphasised

this conclusion in regard to the α1-receptor, showing a decrease in rhabdosphincter electromyography activity after administration of the α1-adrenergic receptor antagonist prazosin, indicating that endogenous NE was being tonically released to maintain urethral motor neuron activity via the activation of α1-adrenoceptors [26]. Another study in humans affirmed the results from the cat studies and found a significant increased contractibility of the EUS towards sacral magnetic stimulation after administration of duloxetine [11]. The results of a recent study with a sneeze-induced incontinence model in rats demonstrated that duloxetine can prevent SUI by facilitating noradrenergic and serotonergic systems in the spinal cord to enhance the sneeze-induced urethral closure mechanism [27]. Furthermore, the results suggested that EUS continence reflexes during sneezing are likely to be regulated by a complex balance among facilitatory 5-HT-receptors, α1- adrenoceptors and inhibitory α2-adrenoceptors [27].

That duloxetine probably has an effect on the human sacral spinal cord, could be demonstrated in this study by a reduced threshold for EUS contractions to TCMS and CLES. This finding is in accordance with previous studies and suggests a facilitatory effect of duloxetine on pudendal sphincter motor neurons in humans [11, 13]. How far there is an effect of duloxetine on supraspinal centres regulating EUS and pelvic floor muscles remains however unclear.

Regarding the hitherto known mechanism of action of duloxetine and PFMT in SUI, it can be assumed that a combination of both will show a synergistic effect in regard to the motor neuron excitability of the EUS. Bearing in mind the hypothesis, that reaching an automatic activation level of the pelvic floor and EUS could be an essential component to regain continence [15], this combined treatment with a significant reduction of the excitability threshold might be a reasonable way to facilitate autonomic reflex contractions in women with SUI. Furthermore, duloxetine taken in course, rather than continously, might improve

compliance with PFMT at the beginning, when PFMT takes some time to show an effect and during relapse [28]. Similar studies as described above in animal models are lacking in humans, but those few neurophysiological investigations in humans performed hitherto are the first steps towards a better understanding of the improved continence with duloxetine found in the past clinical trials. It remains however, a mandatory future challenge to further explore the exact mechanism of action of duloxetine on the human LUT and to validate a combined regimen of PFMT and duloxetine in clinical practice.

There are some limitations of the study. First limitation is the use of healthy subjects without SUI, which restricts the translation of our results on women with SUI.

A second limitation is the small sample size of 10 subjects, which were investigated in a non placebo-controlled non-randomized study. However, this study was planned as a proof of principle study and based on these findings, randomised placebo controlled clinical trials on patients with SUI and age matched healthy subjects are a necessary next step. Our intention was to show the possible mechanism behind a combined treatment of PFMT and duloxetine, which was demonstrated as beneficial in a randomized placebo controlled trial by Ghoniem et al. [14].

A third limitation is that we cannot completely exclude an influence of the first PFMT on M3 and M4. Although we cannot prove that the effect of the first PFMT has been washed out before M3, we would expect at least a similar threshold as after the first PFMT or probably a slight return to baseline. We would not expect a further decrease, as also clinical effects/benefits of PFMT does not further improve on its own without further training. If training is stopped, the effect of the training starts to return to baseline. We therefore attribute the further decrease to duloxetine. One might now argue that the measurements itself might caused a reduction in motor thresholds. That however is highly unlikely as corticomotor threshold determination using single pulse TCMS is a reproducible method with reliable

outcome [29] and we did not observe any influence on the thresholds by the TCMS or CLES itself in pretests, which we performed before the initiation of the study. A facilitation of cortical excitability and modulation of the corticomotor threshold can occur, if repeated TCMS is used for several minutes, whereas low frequency stimulation (1 Hz) suppresses cortical excitability and high frequent stimulation (> 5Hz) increases cortical excitability. Single impulses used for threshold determination however did not show any influence on cortical excitability [30]. Regarding CLES, determination of the bulbocavernosus reflex threshold just 2 times during one measurement and with at least 15 minutes interval between measurements, we would not expect any facilitation.

A forth limitation is that neither CLES nor TCMS reflect of course a physiological stimulation and cannot be compared to or resemble a guarding pelvic floor contraction in daily life. However, CLES and TCMS in combination with a pressure transducer in the EUS are standardized, objective, minimally invasive and very reproducible measures of neuronal excitability in regard to sphincter contractions and therefore useful to investigate therapeutic influences on the efferent pathway of the EUS. If these electrophysiologically measured changes in our study population have a relevant impact on the daily life SUI in affected women has to be further determined in randomized clinical trials.

**Conclusion**

Despite some limitations, the results of this functional study of the female lower urinary tract under the influence of PFMT, duloxetine, and PFMT with duloxetine combined, suggest that the combined treatment causes the lowest excitability thresholds for EUS contractions during CLES and TCMS in young healthy females. Although PFMT and duloxetine alone

significantly lowered the excitability threshold, the best effect could be obtained with the combination of both, which might be a possible explanation for the synergistic mechanism of action of the combined treatment. Reduced excitability thresholds might help to promote continence by amplifying and /or accelerating the EUS reflex contractions during abdominal stress. Although results from this basic study cannot be readily translated to a patient population with SUI, a combined treatment might be worth to be further investigated in patients, in whom PFMT alone is not sufficient enough or who show difficulties with training compliance do to the prolonged onset of effect of the PFMT.

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**Tables**


Table 1: Subject characteristics and raw data of all threshold measurements (TCMS & CLES). The grey highlighted numbers indicate the mean values of the two TCMS and CLES threshold determinations in each measurement, which were used for the statistical calculation. M = measurement, SD = standard deviation, BMI = body mass index.

**Figure legends**


Fig. 1: Experimental paradigm and timing. M = measurement, PFMT = pelvic floor muscle training, EUS = external urethral sphincter, CLES = clitoral electric stimulation, TCMS = transcranial magnetic stimulation.
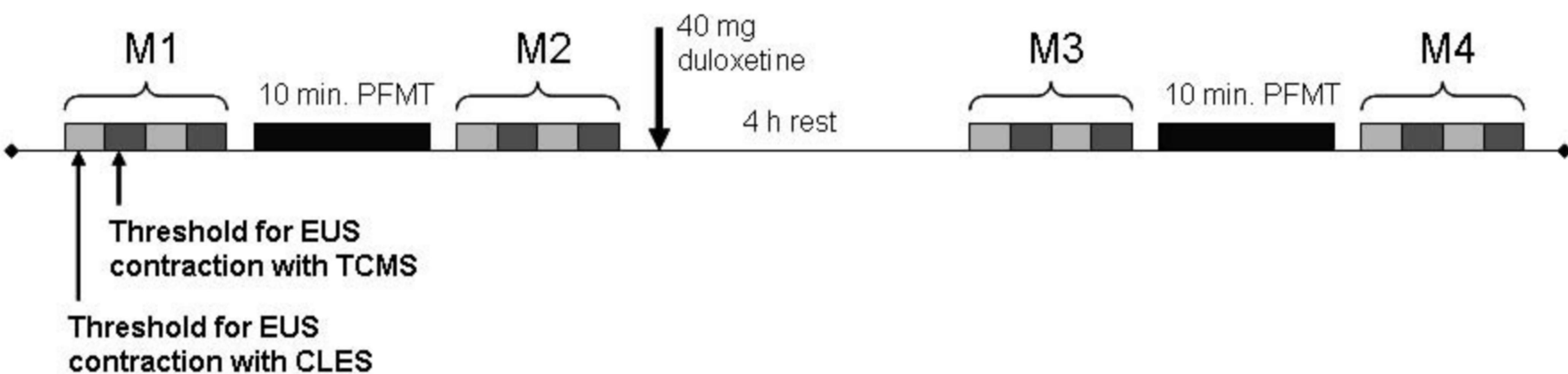

Fig. 2: Fluoroscopy image of one subject, showing the vaginal probe and the transurethral catheter with pressure transducers intravesical and at the external urethral sphincter.


Fig. 3: Motor thresholds for external urethral sphincter (EUS) contractions during clitoral electric stimulation (CLES). The boxplots show minimum, 25% percentile, median, 75% percentile and maximum. Braces indicate significance level between the four different measurements.
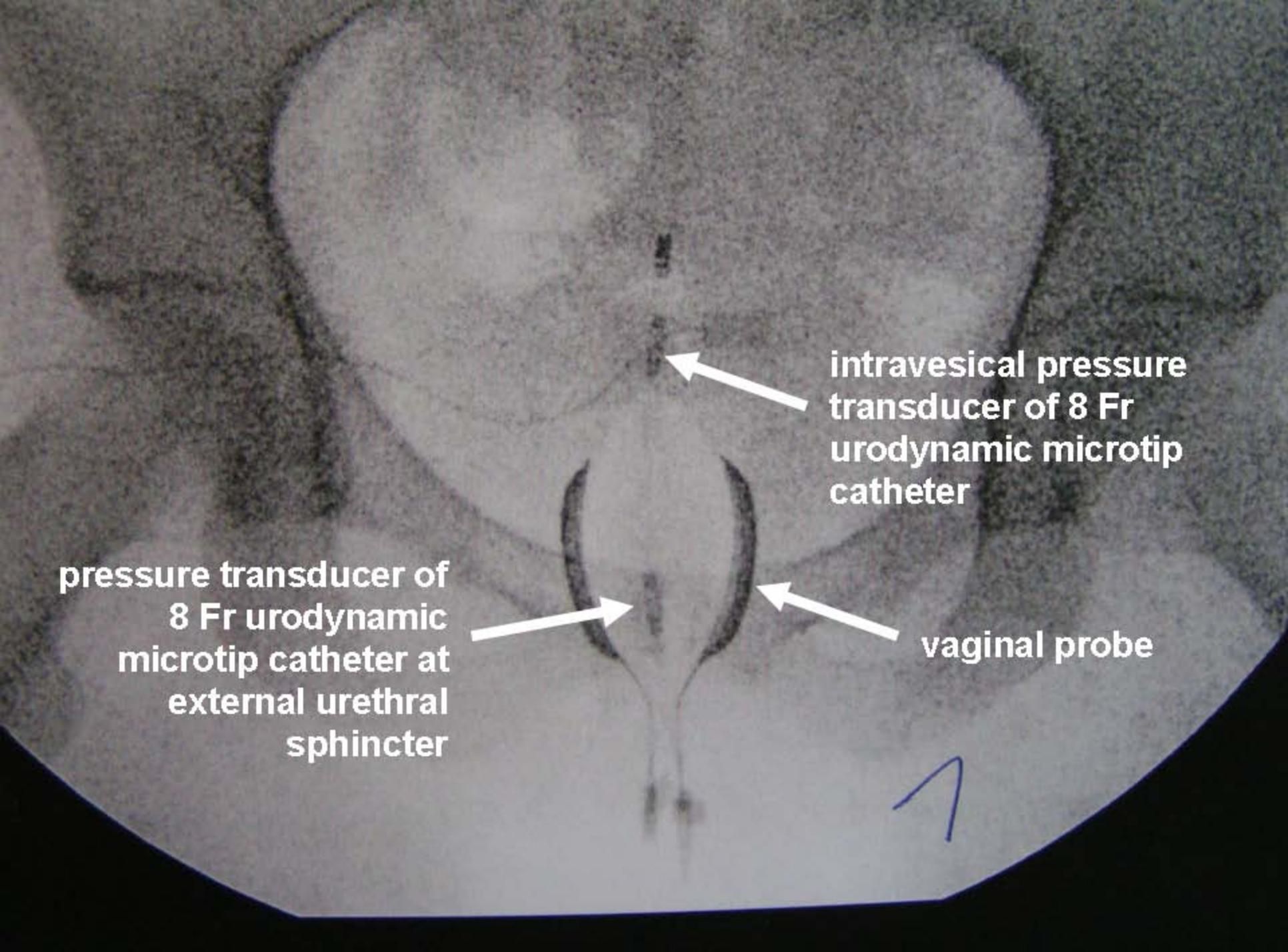

Fig. 4: Motor thresholds for external urethral sphincter (EUS) contractions during transcranial magnetic stimulation (TCMS). The boxplots show minimum, 25% percentile, median, 75% percentile and maximum. Braces indicate significance level between the four different measurements.

| M | Parameter | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean | SD |
|---|-----------|---|---|---|---|---|---|---|---|---|----|------|-----|
| | | | | | | **Subjects** | | | | | | | |
| | Age [years] | 21 | 25 | 29 | 23 | 29 | 25 | 26 | 23 | 21 | 23 | 24.5 | 2.9 |
| | BMI [kg/cm2] | 20 | 19 | 22 | 22 | 22 | 20 | 21 | 23 | 21 | 20 | 21 | 1.2 |
| | Obstetric status | nulli-parous | nulli-parous | nulli-parous | nulli-parous | nulli-parous | nulli-parous | nulli-parous | nulli-parous | nulli-parous | nulli-parous | | |
| | Occupation | student | student | student | student | student | student | student | student | student | student | | |
| | Physical activity level | None of the subjects was performing physical training more than once a week or was professionally involved in sport activities. | | | | | | | | | | | |
| 1 | EUS motor threshold CLES 1 | 28 | 20 | 30 | 10 | 25 | 26 | 27 | 38 | 24 | 20 | 24.8 | 7.3 |
| | EUS motor threshold CLES 2 | 27 | 20 | 30 | 11 | 22 | 26 | 27 | 38 | 24 | 20 | 24.5 | 7.1 |
| | Mean of 1 & 2 | 27.5 | 20 | 30 | 10.5 | 23.5 | 26 | 27 | 38 | 24 | 20 | 24.7 | 7.2 |
| | EUS motor threshold TCMS 1 | 96 | 89 | 99 | 84 | 88 | 80 | 67 | 83 | 79 | 87 | 85.2 | 9.0 |
| | EUS motor threshold TCMS 2 | 99 | 86 | 91 | 82 | 86 | 80 | 67 | 83 | 75 | 79 | 82.8 | 8.7 |
| | Mean of 1 & 2 | 97.5 | 87.5 | 95 | 83 | 87 | 80 | 67 | 83 | 77 | 83 | 84.0 | 8.7 |
| 2 | EUS motor threshold CLES 1 | 21 | 18 | 27 | 10 | 23 | 15 | 25 | 35 | 22 | 13 | 20.9 | 7.3 |
| | EUS motor threshold CLES 2 | 22 | 15 | 27 | 10 | 25 | 16 | 25 | 35 | 24 | 13 | 21.2 | 7.6 |
| | Mean of 1 & 2 | 21.5 | 16.5 | 27 | 10 | 24 | 15.5 | 25 | 35 | 23 | 13 | 21.1 | 7.4 |
| | EUS motor threshold TCMS 1 | 95 | 80 | 94 | 79 | 86 | 73 | 64 | 83 | 75 | 80 | 80.9 | 9.4 |
| | EUS motor threshold TCMS 2 | 95 | 80 | 93 | 76 | 86 | 74 | 63 | 83 | 71 | 76 | 79.7 | 9.9 |
| | Mean of 1 & 2 | 95 | 80 | 93.5 | 77.5 | 86 | 73.5 | 63.5 | 83 | 73 | 78 | 80.3 | 9.6 |
| 3 | EUS motor threshold CLES 1 | 20 | 15 | 19 | 10 | 22 | 16 | 22 | 22 | 21 | 19 | 18.6 | 3.9 |
| | EUS motor threshold CLES 2 | 27 | 14 | 20 | 10 | 18 | 16 | 20 | 20 | 21 | 17 | 18.3 | 4.5 |
| | Mean of 1 & 2 | 23.5 | 14.5 | 19.5 | 10 | 20 | 16 | 21 | 21 | 21 | 18 | 18.5 | 4.0 |
| | EUS motor threshold TCMS 1 | 90 | 82 | 93 | 74 | 83 | 65 | 66 | 82 | 72 | 71 | 77.8 | 9.7 |
| | EUS motor threshold TCMS 2 | 90 | 80 | 90 | 73 | 81 | 63 | 61 | 82 | 72 | 71 | 76.3 | 10.1 |
| | Mean of 1 & 2 | 90 | 81 | 91.5 | 73.5 | 82 | 64 | 63.5 | 82 | 72 | 71 | 77.1 | 9.8 |
| 4 | EUS motor threshold CLES 1 | 22 | 10 | 18 | 8 | 18 | 14 | 22 | 17 | 20 | 13 | 16.2 | 4.8 |
| | EUS motor threshold CLES 2 | 21 | 9 | 19 | 8 | 15 | 14 | 21 | 18 | 20 | 13 | 15.8 | 4.8 |
| | Mean of 1 & 2 | 21.5 | 9.5 | 18.5 | 8 | 16.5 | 14 | 21.5 | 17.5 | 20 | 13 | 16.0 | 4.8 |
| | EUS motor threshold TCMS 1 | 83 | 78 | 86 | 72 | 81 | 60 | 58 | 83 | 61 | 65 | 72.7 | 10.9 |
| | EUS motor threshold TCMS 2 | 80 | 75 | 82 | 69 | 79 | 58 | 55 | 79 | 60 | 65 | 70.2 | 10.1 |
| | Mean of 1 & 2 | 81.5 | 76.5 | 84 | 70.5 | 80 | 59 | 56.5 | 81 | 60.5 | 65 | 71.5 | 10.5 |

M1    10 min. PFMT    M2    40 mg duloxetine    4 h rest    M3    10 min. PFMT    M4

Threshold for EUS contraction with TCMS

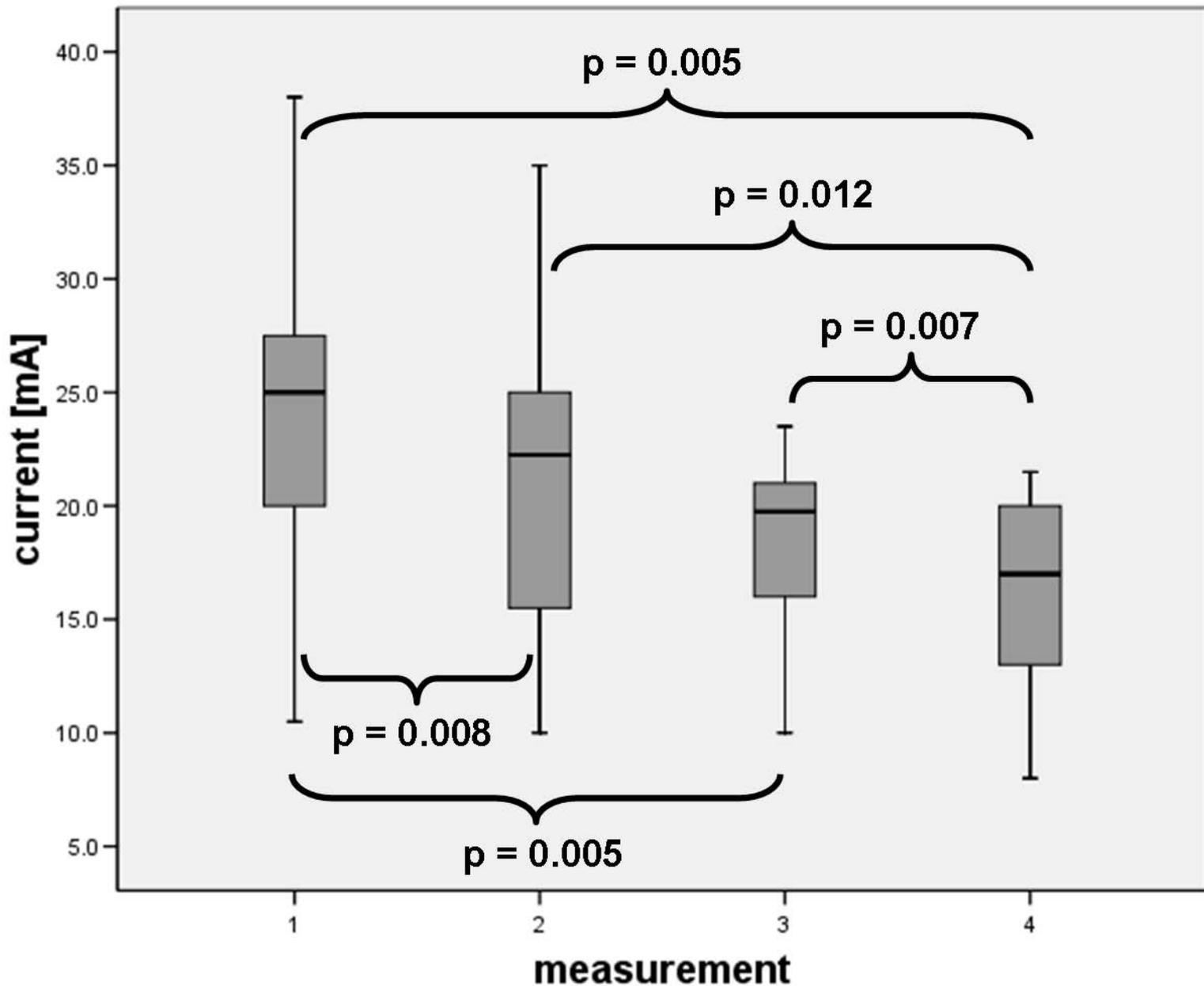Threshold for EUS contraction with CLES

intravesical pressure transducer of 8 Fr urodynamic microtip catheter

pressure transducer of 8 Fr urodynamic microtip catheter at external urethral sphincter

vaginal probe

EUS motor threshold during CES in 10 healthy females

EUS motor threshold during TMS in 10 healthy females