Histological examination of Pelvic Floor Muscle in a Rat Model of Vaginal Delivery

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Introduction

It is well known that transvaginal childbirth weakens the pelvic floor muscles (PFM) and pelvic nerves, leading to stress urinary incontinence (SUI).

A previous report showed that birth trauma with vaginal distention (VD) in rats induced urethral dysfunction and SUI[1)]. Pelvic floor muscle training is recommended as Grade A conservative therapy for SUI. Thus, PFM play important roles in lower urinary tract functions. Peripheral nerve injury causes denervation, which leads to a variety of muscular changes, including muscle atrophy. In skeletal muscle, changes in morphology and fiber type distribution have been reported after nerve transection[2)].

However, it is unknown how PFM and nerve injury after vaginal delivery cause changes in PFM composition. Based on the above, it is predicted that PFM and nerve injury after vaginal delivery will cause PFM muscle atrophy and alter the distribution of muscle fiber types.

The purpose of this study was to examine the histology of PFM in a rat model of VD.

Methods and Materials

Target Animals and Grouping

Twenty-four female Sprague-Dawley rats (211-328 g)

(1) sham-operated group (sham group, n=6)
(2) 1-week after VD (1W group, n=6)
(3) 2-week after VD (2W group, n=6)
(4) 4-week after VD (4W group, n=6).

Urethral pressure measurements

- A microtransducer was placed at 12.5-15 mm from the urethral orifice to measure urethral baseline pressure (UBP) and amplitude of the urethral response to electrical stimulation (A-URE).
- Measurements of A-URE were determined as the maximum pressure change from baseline (cmH_2O) during electrical stimulation (intensity: 1.8 to 2.0 V) of the external abdominal oblique muscle.

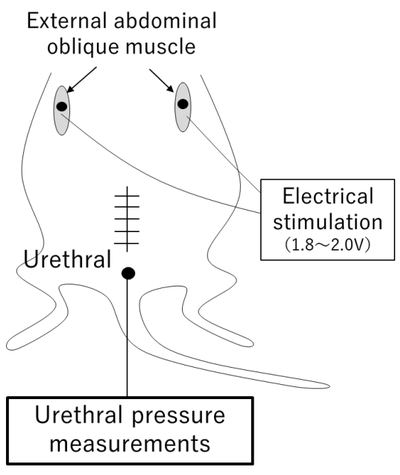


Fig.1 Urethral pressure measurements

Tissue harvesting

- A midline incision was made from the abdomen to the perineal vagina and harvest one side pubococcygeus muscle (Pcm) (Fig. 2).
- Pcm was collected, and its wet weight to body weight was measured.
- For morphological examination, Pcm were frozen in liquid nitrogen and stored at –80° C.

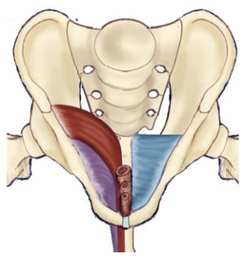


Fig. 2 Drawing of the Pcm (purple) [3)]

Morphological examination

- We used the adenosinetriphosphatas (ATPase, pH=10.2) and succinate dehydrogenase (SDH) activity to identify muscle fiber type (type I, IIa, IIb) distribution.
- Stained images were imported into a personal computer and the cross-sectional area (CSA) (μm^2) of each muscle fiber type was determined.

Parameters

1) UBP, A-URS (cmH_2O)
2) Muscle wet weight (g / body weight)
3) CSA of each muscle fiber (Type I, IIa, IIb) (μm^2)
4) Distribution of type I, IIa, IIb fibers (%)

Statistical analysis

One-way analysis of variance was performed to compare the data among 4 groups. P value of less than 0.05 was regarded to be statistically significant.

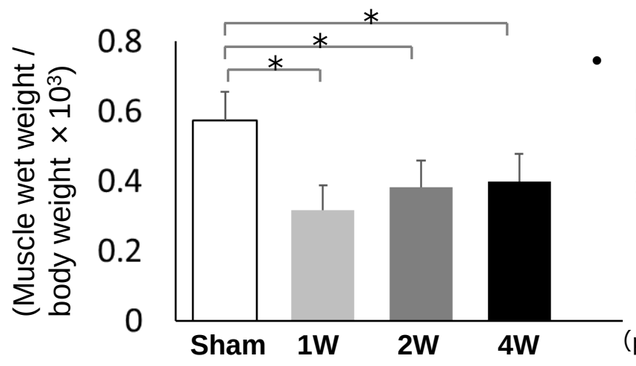
The present study was conducted after being approved by Animal Study Facility Ethics Committee in our institute (No. 19-0062).

Results

UBP, A-URS

- UBP was not significantly different among the four groups.
- In A-URE, 1W group was significantly lower than in the sham, 2W and 4W group ($p < 0.01$, respectively).

Muscle wet weight



- Pcm wet weight was significantly lower in the 1W, 2W, and 4W groups compared to the sham group ($p < 0.01$, respectively) (Fig.3).

(post hoc Turkey test ＊p<0.05)

Fig.3 Pubococcygeus muscle wet weight

CSA of each muscle fiber

- The CSA of type I fibers at Pcm was significantly lower in the 1W, 2W, and 4W groups than in the sham group ($p < 0.05$, respectively) (Fig.4).

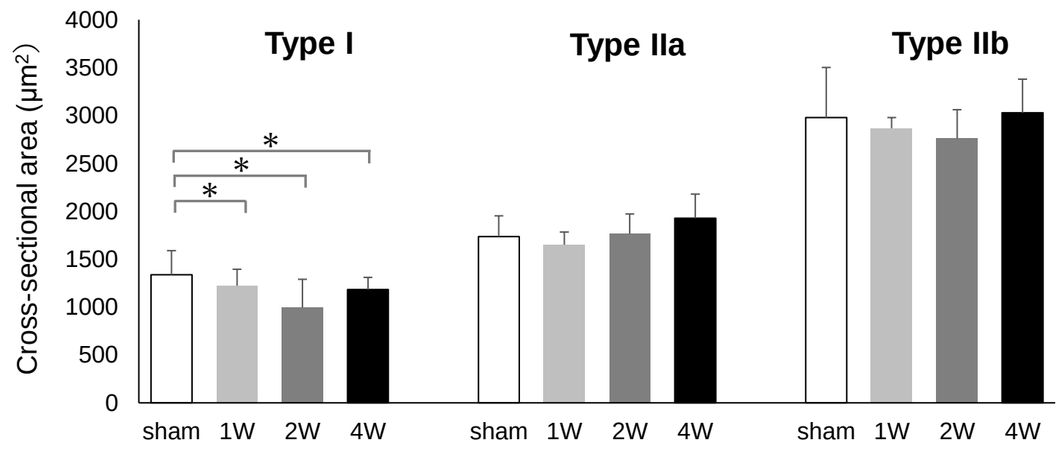
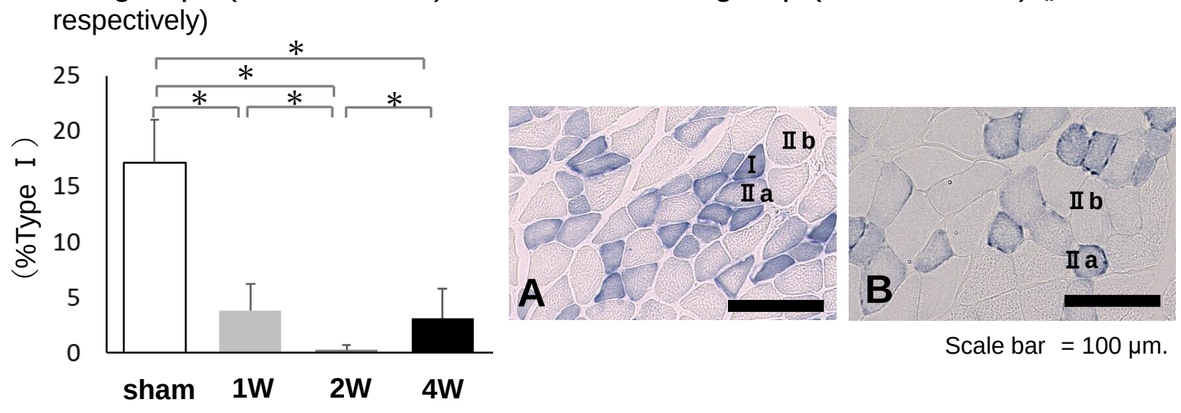


Fig.4 CSA of Type I, IIa, IIb muscle fiber

Distribution of type I, IIa, IIb

- **The type I ratio was significantly lower in the 1W (3.8±2.4 %, p=0.02), 2W (0.3±0.5 %, p=0.01) and 4W (3.1±2.7 %, p=0.02) groups compared to the sham group (17.1±3.4 %). The 2W group was significantly lower than the 1W (p=0.01) and 4W (p=0.04) groups** (Fig 5).
- The ratio of type IIa was not significantly in each group
- The type IIb ration was significantly higher in the 1W (72.8 ± 11.4 %) and 2W groups (73.0 ± 9.9 %) than in the sham group (55.2 ± 3.6 %) ($p < 0.01$, respectively)



Scale bar = 100 μm.

Fig. 5 Distribution of type I; A: sham group, B: 2 week after vaginal distention (VD) (2W group). Type I (dark blue), IIa (neutral blue), IIb (pale blue).

Discussion

The muscle wet weight of Pcm was significantly lower in the 1W, 2W, and 4W groups than in the sham group, with the lowest value in the 1W group. In addition, Type I CSA was significantly lower in the 1W, 2W, and 4W groups than in the sham group. In the previous report, a decrease in muscle wet weight due to denervation was observed from 1-week postoperatively[4)], and a significant decrease in CSA was observed in the denervation-induced muscle atrophy[5)], especially in Type I. Therefore, it is possible that significant muscle atrophy in Type I fibers occurred afterVD. In general, in a rat model of vaginal delivery after VD, SUI occur at 4 weeks postoperatively due to decreased A-URE, but incontinence improve at 2 weeks after VD[6)].

This study also showed significant improvement in A-URE at 2 weeks postoperatively, suggesting that urethral function was restored but PFM function was not. The distribution of muscle fiber type I was significantly lower in the 1W, 2W and 4W groups than in the sham group, with the lowest value in the 2W group. Experiments confirming changes in muscle composition of rat soleus muscle after sciatic nerve transection show " fast-twitch muscle" with a decrease in type I and a relative increase in the proportion of type II muscle[7)]. In addition, muscle atrophy has a stronger effect on Type I (slow muscle) fibers.

This leads to fast-twitch muscle and reduced contractility, which may contribute to the persistent loss of contractility in PFM.

Conclusions

Muscle atrophy and changes in muscle composition (fast-twitch muscle) due to reductions in PFM muscle wet weight and CSA were observed in the VD rat model, even after urinary incontinence of SUI had improved.

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Disclosure statement

Conflict of Interest (COI) of the principal presenter: No potential COI to disclose

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