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Proportions of fiber types in the external urethral sphincter of young nulliparous and old multiparous rabbits

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Summary. The proportions of fast and slow myosin molecules in specimen's of the external urethral sphincter (EUS) from 6 young nulliparous (6-month-old) and 6 old multiparous (2-year-old) rabbits were studied using myosin heavy chain electrophoresis. The percentages of fast and slow myosin molecules were $80.4 \pm 4.5\%$ and $19.6 \pm 4.5\%$ in the EUS from nulliparas, and $68.7 \pm 6.3\%$ and $31.3 \pm 6.3\%$ in the EUS from multiparas. The difference between the two groups was significant ($P < 0.01$). We suggest that a selective decrease in the volume of type 2 (fast) muscle fibers and/or conversion of type 2 to type 1 (slow) muscle fibers had taken place in the EUS of old multiparas. The proportional change in the constituent muscle fibers of the EUS with aging may play a role in human genuine stress incontinence.

Key words: Aging – Atrophy – External urethral sphincter – Female rabbit – Myosin heavy chain – SDS-PAGE

It is generally considered that genuine stress incontinence is more common among old multiparous women than among young nulliparous women [5]. Recent studies show that loss of strength and atrophy of human skeletal muscle with aging are attributable to selective atrophy of type 2 (fast) muscle fibers [1, 15, 18]. The present study was designed to examine the difference in the proportions of fast and slow myosin molecules in the external urethral sphincter (EUS) between young nulliparous and old multiparous female rabbits.

Materials and methods

Localization of female rabbit EUS

As shown in Fig. 1, in female rabbits the bladder and vagina open into a single lumen, i.e., the vestibulum, which opens in the perineum ventral to the rectal vent. This female urogenital anatomy, completely different from that of the human female, in which there are separate openings for the urethra and vagina, is similar to that in many other mammals such as the dog and the cat [8]. These female animals thus preserve the structure of the urogenital sinus. The female rabbit's EUS encircles both the bladder neck and the distal end of the vagina. There are no EUS muscle fibers between the bladder neck and the vagina.

Sample collection and preparation

Six young nulliparous (6-month-old) and 6 old multiparous (tri- or quadriparous, 2-year-old) female Japanese white rabbits (body weight 3.0 kg) were killed by rapid injection of pentobarbital sodium solution into an ear vein. The bladder, vestibulum and vagina were extracted en bloc and stored at −80°C. The portion of the frozen specimen containing the EUS was cut transversely into serial sections about 0.3 mm thick and stored in 50% glycerol, 50% relaxing buffer (RB) [23] at −20°C for 2 weeks. Glycerinated muscle fibers were dissected out from these sections in RB on crushed ice

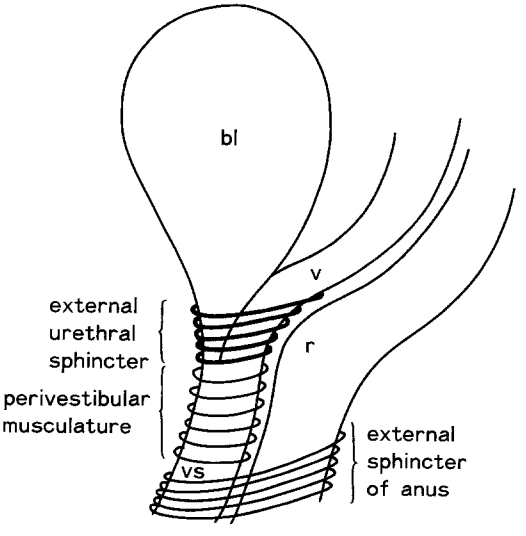

Fig. 1. Schematic illustration of the lower urinary and rectal systems of female rabbit. *bl,* Bladder; *v,* vagina; *vs,* vestibulum; *r,* rectum

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Table 1. Wet weights of isolated muscle fibers of the external urethral sphincter from 6 young nulliparous (6-month-old) and 6 old multiparous (2-year-old) rabbits

	Weight/(mg) Mean ± SD (range)
Young rabbits ($n=6$)	105.9 ± 21.0 (71.5–133.0)
Old rabbits ($n=6$)	97.5 ± 29.4 (59.3–141.3)

The difference between the two groups is not significant

Table 2. The proportions of fast and slow myosin heavy chains (MHC) in external urethral sphincter samples from 6 young nulliparous and 6 old multiparous rabbits

	Fast MHC (%) Mean ± SD (range)	Slow MHC (%) Mean ± SD (range)
Young rabbits ($n=6$)	80.4 ± 4.5 (73.2–87.3)	19.6 ± 4.5 (12.7–26.8)
Old rabbits ($n=6$)	68.7 ± 6.3 (60.1–77.1)	31.3 ± 6.3 (22.9–39.9)

The difference between the two groups is significant ($P<0.01$)

under a dissection microscope. The isolated muscle fibers were homogenized in 3 volumes of Hasselbach-Schneider solution [13] at 4°C with a micro-glass homogenizer for 16 min as centrifuged at 600 *g* for 15 min. The supernatant was used as a sample of crude myosin for electrophoresis. Protein concentration was determined by Bradford's method [3].

Electrophoresis

SDS-polyacrylamide gel electrophoresis (PAGE) analyzing myosin heavy chains [6] was performed. The separating gel was 6% and the stacking gel 4%; both gels contained 40% (v/v) glycerol. Ten micrograms of sample was applied to each well of the gels and electrophoresis carried out at 120 V for 12 h. The gels were stained and destained as previously reported [23].

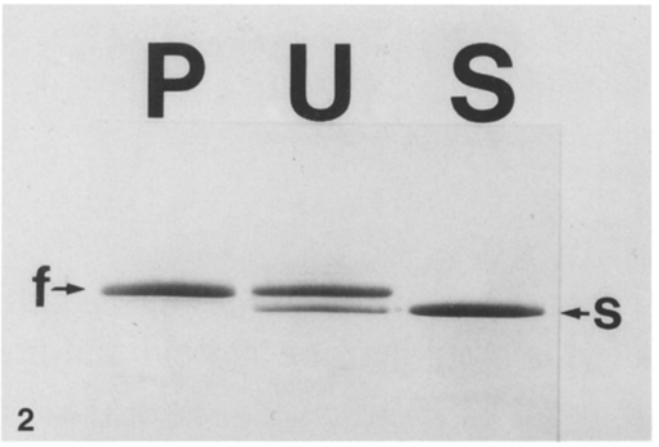
The molar ratio of fast myosin heavy chains to slow myosin heavy chains

The bands of fast and slow myosin heavy chains were cut out from the gel following staining and destaining. Coomassie Brilliant Blue R was extracted from the gel pieces with pyridine, and the protein concentration determined by the absorbance at 605 nm according to Fenner [11], bovine serum albumin being used as the standard.

Results

Wet weights of isolated glycerinated EUS muscle fibers from 6 young nulliparous and 6 old multiparous rabbits are shown in Table 1. Mean weight of EUS from young nulliparas was 105.9 ± 21.0 mg and that from old multiparas was 97.5 ± 29.4 mg. The difference between the two groups was not significant.

Figure 2 shows SDS-PAGE gel of myosin heavy chains from psoas, soleus and EUS muscles of rabbit. In psoas,



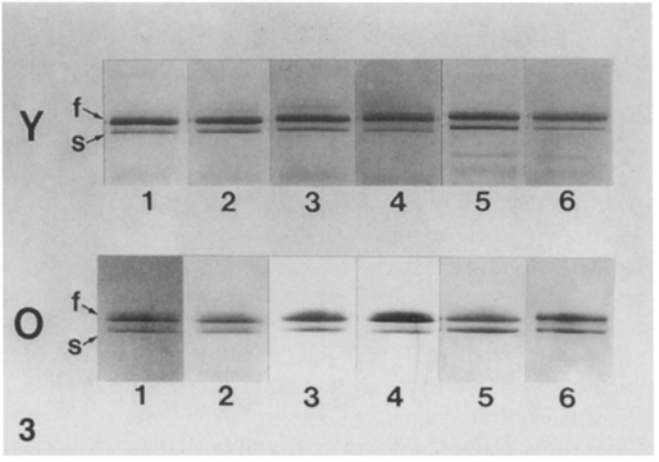


Fig. 2. SDS-PAGE gel of myosin heavy chains from psoas, external urethral sphincter and soleus. In the lane for psoas (*P*), a muscle predominantly composed of fast fibers, a band of fast myosin heavy chains (*f*) is seen. In that of soleus (*S*), a muscle mainly composed of slow fibers, a band of slow myosin heavy chains (*s*) is present. In the lane for the external urethral sphincter (*U*) bands of both fast and slow myosin heavy chains are seen

Fig. 3. SDS-PAGE gels of myosin heavy chains from the external urethral sphincters of 6 young nulliparous rabbits (*upper line*) and 6 old multiparous rabbits (*lower line*). In all specimens fast (*f*) myosin heavy chains predominate over slow (*s*) chains

the constituent fibers of which are almost all fast type, there is only a band of fast myosin heavy chains. In soleus, which is composed almost entirely of slow twitch fibers, a single band of slow myosin heavy chains is present. In EUS, which has almost equal proportions of fast and slow twitch fibers, two bands corresponding to fast and slow myosin heavy chains are seen.

Figure 3 shows SDS-PAGE gels of myosin heavy chains from EUS of 6 young nulliparous and 6 old multiparous rabbits. In all specimens two bands corresponding to fast and slow myosin heavy chains are observed. The fast myosin heavy chains are predominant in all cases.

The proportions of fast and slow myosin heavy chains in EUS in 6 young nulliparous and 6 old multiparous rabbits are presented in Table 2. The proportions were

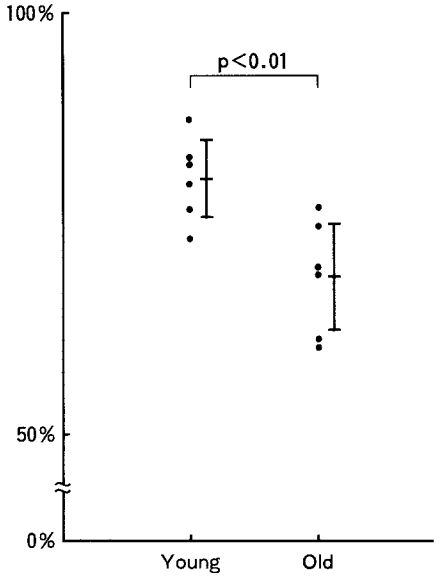


Fig. 4. The proportion of fast myosin heavy chains in external urethral sphincter samples from 6 young nulliparous and 6 old multiparous rabbits. The difference between the two groups is significant ($P < 0.01$)

calculated from the molar ratio of fast to slow myosin heavy chains in each EUS specimen. The molar ratio of fast to slow myosin heavy chains was analyzed with 16 electrophoretic lanes per sample. The proportion of fast myosin heavy chains in young nulliparas ranged from 73.2% to 87.3% with a mean of 80.4%. That in old multiparas ranged from 60.1% to 77.1% with a mean of 68.7%. The difference between the two groups was significant ($P < 0.01$) (Fig. 4).

Discussion

Adult mammalian voluntary muscle is generally composed of three fiber types with different ATPase activities: type 1 is a truely fatigue-resistant slow twitch fiber, type 2A is a relatively fatigue-resistant fast twitch fiber, and type 2B is a fatigue-sensitive fast twitch fiber [16]. It is known that myosin isozymes in fast and slow twitch fibers are different [10, 12].

One molecule of myosin is composed of two myosin heavy chains (HC) and four myosin light chains (LC). Studies using SDS-electrophoresis on single muscle fibers revealed that myosin heavy chains from type 1, 2A and 2B fibers are distinct; they are classified as HC1, HC2A and HC2B respectively [20]. In this study we could not separate HC2A and HC2B and had a single HC2 band on electrophoretic analysis because we used whole muscle extracts. The proportions of HC1 and HC2 in the EUS whole muscle extract represent the proportions of slow and fast myosin HC molecules in each EUS.

We found that the proportion of fast myosin molecules in the EUS was lower in old multiparas than in young nulliparas. Given that there was not significant difference between the weights of EUS of the two groups, there are three possible explanations for this change in the proportions of myosin molecules with age: an increase in the volume of type 1 fibers, a decrease in the volume of type 2 fibers, or both.

Hypertrophy of type 1 fibers and/or a conversion of type 2 to type 1 fibers take place with prolonged training [2, 17, 21]. Since our animals had been reared in a laboratory cage without a special training program, an increase in the volume of type 1 fibers by this mechanism would be unlikely.

Many researchers have ascribed the predominance of type 1 fibers in the skeletal muscles of elderly humans and animals to a selective atrophy and decrease in type 2 fibers [1, 15, 18]. Muscular structural changes suggesting neurogenic disorders are known to increase with age [15]. Several studies on human anterior horn cells have shown a significant decrease in motor units after the age of 60 years [4]. Selective degeneration of the largest and most rapidly conducting motoneurons (type 2) and/or transformation of type 2 fibers to type 1 followed by nonselective loss of motor units has been suggested [9].

The increase in the proportion of slow myosin molecules in the EUS of old multiparous rabbits, as confirmed by the present study, would thus probably be attributable to a selective decrease and/or atrophy of type 2 fibers and/or transformation of type 2 fibers to type 1 by denervation with aging.

Some researchers [22] have described increasing neurological abnormalities of the pelvic floor musculature in human multiparas with increasing numbers of vaginal deliveries, and have suggested that the peripheral nerves of the pelvic floor musculature sustain damage during vaginal delivery. It remains to be established whether such damage cause the change in the proportion of EUS myosins demonstrated in the present study.

The motoneurons of slow twitch type 1, fast twitch type 2A and fast twitch type 2B fibers are different from one another: the motoneuron of a type 1 fiber is small, low-threshold and slow conducting; that of type 2B is large, high-threshold and fast conducting; and that of type 2A is intermediate in size, threshold and speed of conduction. During low-intensity exercise, when the stimulus from the central nervous system is weak, only type 1 fibers are recruited. As the intensity of exercise rises, type 2A and then type 2B fibers are recruited by the stronger stimulus. This phenomenon is called the size principle [19] and is common in mammalian voluntary muscles.

It is considered that the recruitment of motor units in the contraction of the EUS occurs in the following manner. During urinary storage the EUS contracts continuously, recruiting only the motor units of fatigue-resistant type 1 fibers by weak nerve stimuli. When stopping the urinary stream, or when there is a sudden increase in the intravesical pressure during stress and/or urgency conditions, the motor units of fatigue-sensitive type 2 fibers are recruited by the stronger nerve stimuli to effect short strong contractions. A decrease in the type 2 fibers of the EUS would mean the muscle could not

perform short strong contractions adequately under stress conditions. The decrease and/or atrophy of type 2 fibers in the EUS may therefore play a role in genuine stress incontinence in women.

Resistant training of the lower extremities in elderly women is reported to lead to type 2 fiber hypertrophy in the vastus lateralis muscle [7]. Our results suggest that pelvic floor training [14], which is effective for the treatment of genuine stress incontinence, may produce type 2 hypertrophy in the EUS as well as increased strength of the pelvic floor musculature.

References

1. Aniansson A, Grimby G, Hedberg M, Krotkiewski M (1981) Muscle morphology, enzyme activity and muscle strength in elderly men and women. Clin Physiol 1:73
2. Baumann H, Jäggi M, Soland F, Howald H, Schaub MC (1987) Exercise training induces transitions of myosin isoform subunits within histochemically typed human muscle fibres. Pflügers Arch 409:349
3. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal Biochem 72:248
4. Brown WF (1972) A method for estimating the number of motor units in thenar muscles and the changes in motor unit count with aging. J Neurol Neurosurg Psychiatry 35:845
5. Burgio KL, Matthews KA, Engel BT (1991) Prevalence, incidence and correlates of urinary incontinence in healthy, middle-aged women. J Urol 146:1255
6. Carraro U, Catani C (1983) A sensitive SDS-PAGE method separating myosin heavy chain isoforms of rat skeletal muscles reveals the heterogeneous nature of the embryonic myosin. Biochem Biophys Res Commun 116:793
7. Charette SL, McEvoy L, Pyka G, Snow-Harter C, Guido D, Wiswell RA, Marcus R (1991) Muscle hypertrophy response to resistance training in older women. J Appl Physiol 70:1912
8. Cragie EH (1948) The urogenical system. In: Cragie EH (ed) Bensley's practical anatomy of the rabbit. University of Toronto Press, Toronto, p 122
9. Edström L, Larsson L (1987) Effects of age on contractile and enzyme-histochemical properties of fast- and slow-twitch single motor units in the rat. J Physiol 392:129
10. Fardeau M, Godet-Guilain J, Tome FMS, Carson S, Whalen RG (1978) Congenital neuromuscular disorders: a critical review. In: Aguayo AJ, Karpati G (ed) Current topics in nerve and muscle research. Excerpta Medica, Amsterdam, p 174
11. Fenner C, Traut RR, Mason Dt, Wikman-Coffelt J (1975) Quantification of Coomassie Blue stained proteins in polyacrylamide gels based on analysis of eluted dye. Anal Biochem 63:595
12. Fitzsimons RB, Hoh JFY (1981) Isomyosins in human type 1 and type 2 skeletal muscle fibres. Biochem J 193:229
13. Hasselbach W, Schneider G (1951) Der L-myosin and Aktingehalt des Kaninchenmuskels. Biochem Z 321:461
14. Kegel AH (1948) Progressive resistance exercise in the functional restoration of the perineal muscles. Am J Obst Gynecol 56:238
15. Oertel G (1986) Changes in human skeletal muscles due to aging. Acta Neuropathol (Berl) 69:309
16. Padykula HA, Herman E (1955) Factors affecting the activity of adenosine triphosphatase and other phosphatases as measured by histochemical techniques. J Histochem Cytochem 3:161
17. Peter WW, Kelly FJ, Goldspink DF, Goldspink G (1982) Exercise-induced morphological and biochemical changes in skeletal muscle of the rat. J Appl Physiol 53:1144
18. Poggi P, Marchetti C, Scelsi R (1987) Automatic morphometric analysis of skeletal muscle fibers in the aging man. Anat Rec 217:30
19. Saltin B, Gollnick PD (1983) Skeletal muscle adaptability: significance for metabolism and performance. In: Peachey LD, Adrian RH, Geiger SR (ed) Handbook of Physiology, Sec. 10, Skeletal Muscle. Williams & Wilkins, Baltimore, p 555
20. Schmitt TL, Pette D (1990) Correlations between troponin-T and myosin heavy chain isoforms in normal and transforming rabbit muscle fibers. In: Pette D (ed) The dynamic state of muscle fibers. de Gruyter, Berlin New York, p 293
21. Souminen H, Heikkinen E, Liesen H, Michel D, Hollman W (1977) Effects of 8 weeks' endurance training on skeletal muscle metabolism in 56-70-year-old sedentary men. Eur J Appl Pysiol 37:173
22. Swash M, Snooks SJ, Henry MM (1985) A unifying concept of pelvic floor disorders and incontinence. J R Soc Med 78:906
23. Tokunaka S, Okamura K, Fujii H, Yachiku S (1990) The proportions of fiber types in human external urethral sphincter: electrophoretic analysis of myosin. Urol Res 18:341