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The proportions of fiber types in human external urethral sphincter: electrophoretic analysis of myosin

S. Tokunaka, K. Okamura, H. Fujii, and S. Yachiku

Department of Urology, Asahikawa Medical College, Asahikawa, Japan

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Summary. The proportions of fast and slow myosin molecules in external urethral sphincter specimens from ten urodynamically normal male bladder carcinoma patients were estimated from the contents of fast and slow myosin light chains in two-dimensional electrophoretic gels. The percentages of fast and slow myosin molecules ranged from 5.0% to 61.4% with a mean of 35.5% and from 38.6% to 95.0% with a mean of 65.5% respectively. It is therefore concluded that the human external urethral sphincter is composed of both fast and slow muscle fibers as well as other voluntary muscles. The human external urethral sphincter is considered to be a highly fatigue-resistant muscle with a very high proportion of slow muscle fibers. In the cases studied so far, there is a great diversity in the proportions of fast and slow myosin molecules; the reason for this remains unknown.

Key words: External urethral sphincter – Myosin – Electrophoresis – Male – Human

Mammalian striated muscle fibers, including human ones, are divided broadly into fast- and slow-twitch muscle fibers. Fast-twitch muscle fiber can contract quickly but easily fatigues, while slow-twitch muscle fiber contracts slowly but is a fatigue-resistant tonic type. The motoneurons of fast- and slow-twitch muscle fibers are also known to be different from each other [11]. Individual muscle is composed of both fast- and slow-twitch muscle fibers, and the proportions of fiber types are characteristically distinct from muscle to muscle, depending on the function of the muscles concerned [10].

It seems to be necessary to investigate the proportion of fiber types in human external urethral sphincter (EUS) in order to understand the functions of the lower urinary tract and/or its malfunctions in neurogenic disorders. This is because muscle fiber types can change, for example with denervation [15], cross-reinnervation [22] and continuous electrical stimulation [22]. To date there has been little research on the muscle fiber types of the human EUS [8, 19].

In this paper we report the results of biochemical analysis of the proportion of muscle fiber types in human EUS.

Materials and methods

Sample collection and preparation

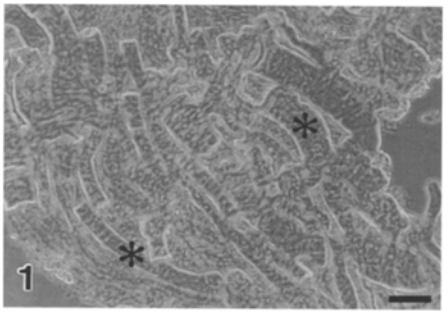
Human male urethras were taken from cystourethrectomy specimens obtained during surgery for carcinoma of the bladder. Patient age ranged from 53 to 80 years, with a mean age of 63.5 years, and all the patients had had normal urodynamics. The urethral portion containing EUS muscle (the prostate and the membranous urethra) was processed and stored in 50% glycerol and 50% relaxing buffer (RB) as previously reported [20]. The EUS muscle layer was dissected from the glycerinated urethra under a dissecting microscope in RB on crushed ice (Figs. 1, 2).

Preparation of sample for two-dimensional electrophoresis from glycerinated EUS muscle

Glycerinated muscle was rinsed with RB and was homogenized in lysis buffer with a microglass homogenizer. The homogenate was centrifuged at 600 *g* for 10 min, and the supernatant was used as the sample. Protein concentration was determined by Bradford's method [3], with bovine serum albumin as standard.

Two-dimensional electrophoresis

Two-dimensional electrophoresis was performed according to the procedure of O'Farrell [13]. One hundred micrograms of sample was applied to isoelectric focusing (IEF) gels. IEF was carried out for 12 h at 400 V, and finally for 1 h at 800 V without a prerun. The IEF gels from the one dimension were loaded onto 15% acrylamide slab gels with a 2.5 cm 4.5% acrylamide stacking gel. Electrophoresis was performed at 5 mA for 2 h and then 20 mA until completion. Gels were stained overnight with 0.13% Coomassie Brilliant Blue R in 45% ethanol/9.2% acetic acid at room temperature, and destained in 5% ethanol/7.5% acetic acid gently shaken at 37°C.



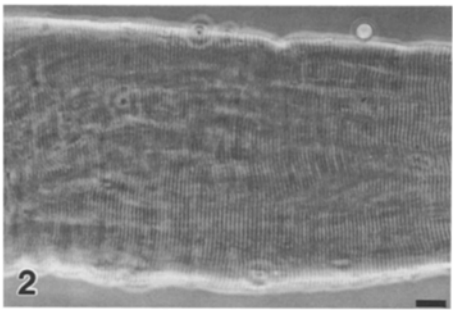


Fig. 1. Phase-contrast micrograph of glycerinated human external urethral sphincter. Multiple myofibrils (*asterix*) are scattered in the connective tissue.
Scale bar = 100 μm

Fig. 2. Phase-contrast micrograph of glycerinated human external urethral sphincter. Characteristic cross-striations are seen in myofibril. Scale bar = 50 μm

Molar ratio of fast myosin light chain 2 to slow myosin light chain 2

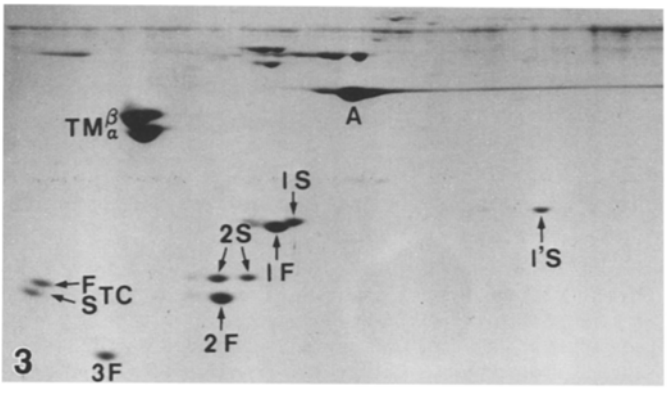
The spots of fast and slow myosin light chain 2 were cut out from the two-dimensional gel following staining and destaining as above. Coomassie Brilliant Blue R was extracted from the gel pieces with pyridine, and the protein concentration was determined with the absorbance at 605 nm, bovine serum albumin being used as standard according to Fenner [5].

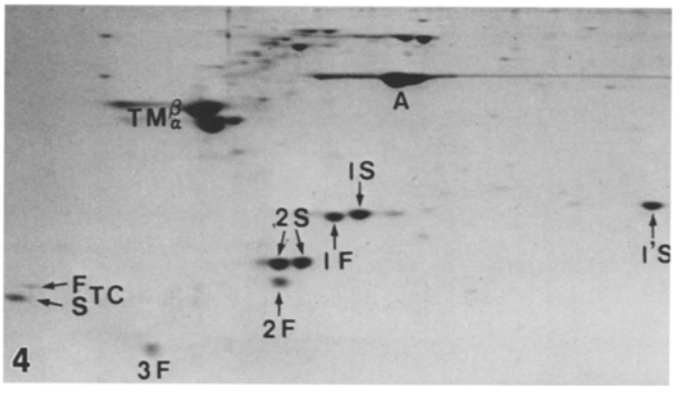
Results

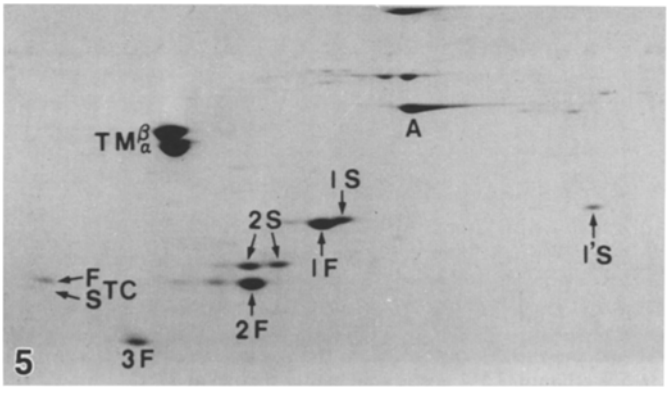
In samples for two-dimensional electrophoresis, myosin molecules are disintegrated into their components, i.e., myosin heavy and light chains. Myosin molecules and/or myosin heavy and light chains from fast-twitch muscle fiber and those from slow-twitch muscle fiber are known to be different. Fast and slow myosin heavy chains cannot be analyzed by two-dimensional electrophoresis because their large size means that they hardly enter IEF gels [11].

In two-dimensional electrophoretograms, fast myosin light chains (LC-1F, 2F and 3F) and slow myosin light chains (LC1S, 1'S and 2S) are situated in different positions. Troponin C, which is one of the muscle regulatory proteins, is also separated into fast and slow types [2, 4, 7]. The pattern of the two-dimensional electrophoretogram of human EUS is similar to that of other human striated muscles, such as rectus abdominis muscle, as previously reported [21].

Figures 3–5 show three different patterns of two-dimensional electrophoretograms of human EUS. In these electrophoretograms from different cases (Fig. 3, case 4 in Table 1; Fig. 4, case 10; and Fig. 5, case 3) both fast myosin light chains 1F, 2F and 3F, and slow myosin light chains 1S, 1'S and 2S are seen. Fast and slow forms of troponin C also exist. In case 4 (Fig. 3), almost equal amounts of fast and slow myosin light chain components are seen, with equal amounts of fast and slow troponin C. In case 10 (Fig. 4), in contrast the amount of slow myosin light chains is more prominent than that of fast myosin







Figs. 3–5. Three different patterns of two-dimensional electrophoretograms of human external urethral sphincter. *1F, 2F* and *3F* = fast myosin light chains; *1S, 1'S* and *2S* = slow myosin light chains; *FTC* = fast troponin C; *STC* = slow troponin C; *αTM* = βTM = tropomyosin; *A* = actin

Fig. 3. Case 4 in Table 1. Amounts of *1F, 2F* and *3F* and *1S, 1'S* and *2S* components are almost equal. *FTC* and *STC* are stained equally.
Fig. 4. Case 10 in Table 1. More *1S, 1'S* and *2S* exist than *1F, 2F* and *3F*, especially between 2S and 2F. *STC* is predominant over *FTC*.
Fig. 5. Case 3 in Table 1. Predominantly *1F, 2F* and *3F* are present, with small amounts of *1S, 1'S* and *2S*. *FTC* is more prominent than *STC*

Table 1. The proportions of fast and slow myosin molecules in human external urethral sphincter muscle (means ± 1SD; $n = 9$)

Case no.	Age (years)	Fast myosin	Slow myosin
1 SI	53	16.5 ± 9.5%	83.5 ± 9.5%
2 AM	53	29.2 ± 1.9%	70.8 ± 1.8%
3 TF	54	61.2 ± 2.9%	38.8 ± 2.0%
4 TN	61	49.4 ± 2.8%	50.6 ± 2.8%
5 ST	63	5.0 ± 2.0%	95.0 ± 2.0%
6 SS	63	16.9 ± 1.7%	83.1 ± 1.7%
7 MH	67	26.6 ± 1.8%	73.4 ± 1.8%
8 YA	70	61.4 ± 5.2%	38.6 ± 5.2%
9 SS	71	60.2 ± 0.5%	39.8 ± 0.5%
10 SM	80	18.2 ± 4.8%	81.8 ± 4.8%
Mean		35.5%	65.5%

light chains, and in case 3 (Fig. 5) predominantly fast myosin light chains are present, with small amounts of slow myosin light chains. With respect to troponin C, the slow type is more prominent than the fast in case 10 (Fig. 4) and the proportions are reversed in case 3 (Fig. 5).

One molecule of myosin has two myosin heavy chains and four myosin light chains, and two of the four myosin light chains are LC-2 (LC-2F and LC-2S in fast and slow myosin molecules, respectively) [11]. This means that the ratio of LC-2F to LC-2S represents the ratio of fast to slow myosin molecules [9].

Table 1 shows the proportions of fast and slow myosin molecules in EUS in ten cases; in each case, the proportions were calculated from the molar ration of LC-2F to LC-2S in samples for two-dimensional electrophoresis. The molar ratio of LC-2F to LC-2S was analyzed with nine gels per each sample. The percentage of fast myosin molecules ranges from 5.0% to 61.4%, with a mean of 35.5%. That of slow myosin molecules ranges from 38.6% to 95.0%, with a mean of 65.5%. No relationship is seen between patient age and the proportion of fiber types in the EUS ($P > 0.05$).

Discussion

Mammalian striated muscle is composed of muscle fibers. Muscle fibers are divided into three fiber types, namely type 1, 2A and 2B histochemically. Type 1 is oxidative, fatigue-resistant, slow-twitch muscle fiber. Both type 2A and 2B are glycolytic oxidative, fatigue-sensitive, fast-twitch muscle fibers, while type 2A is slightly more fatigue-resistant than type 2B [14].

During the last decade, type 1 fiber has been found to have slow specific isozymes of the constituent proteins; myosin and its components (myosin heavy and light chains) and troponin, and type 2A and 2B fibers have fast specific isozymes [2, 4, 6, 7]. Muscular fiber types are therefore identifiable by biochemical protein analysis using mainly electrophoretic techniques, as shown in this report.

The proportion of fiber types in each muscle is different from others and represents its characteristics and function. For example, in humans, soleus muscle, which is situated beside the tibia and holds tonic contraction for hours for the maintenance of posture, is made up of 80% type 1 and 20% type 2 fibers. The orbicularis oculi muscle, which has to contract quickly for blinking, on the other hand, is made up of 15% type 1 and 85% type 2 fibers [10].

In many previous studies on physiological twitch speed of the EUS, it was described as a slow muscle for the maintenance of urinary continence. To date, histochemical and/or biochemical studies on EUS with special emphasis on the proportions of fiber types are quite few. Bazeed et al. [1] reported that the canine EUS was composed of 35% type 1 and 65% type 2 fibers. We have shown previously that the rabbit EUS is made up of 12% type 1 and 88% type 2 fibers [20]. The proportions of fiber types in the same muscle in different mammalian species are known to vary, so the different proportions in canine and rabbit EUS can be attributed to species specificity.

Only three studies on the proportions of fiber types in human EUS have been reported up to now, and there is some controversy. Gosling et al. [8] described the human EUS as true slow muscle composed of purely type 1 fibers following their histochemical and electron microscopic analysis of nine urethral specimens obtained by cystourethrectomy. Schrøder and Reske-Nielsen [19], in contrast reported the coexistence of type 1 and 2 fibers in various proportions in the EUS from three autopsy cases. We found both slow and fast isozymes of myosin in samples from EUS from two cystourethrectomy cases [21].

Fast and slow myosin light chains are specific to fast-twitch (type 2A and 2B) and slow-twitch (type 1) muscle fibers, respectively, except for LC1F [9]. LC1F is seen not only in fast-twitch fibers but also in slow-twitch fibers in human muscle, as previously reported. Since we found fast and slow myosin light chains in all EUS samples investigated, we reconfirmed that the human EUS is composed of fast- and slow-twitch muscle fibers, as are other voluntary muscles. Although to date the proportion of fiber types, which reflects the characteristic of individual muscle, has been determined as the ration of type 1 to type 2 fibers, using ATPase staining, the ratio of fast to slow myosin molecules, as determined in this study, is more appropiate as a reflection of the functional nature of muscle.

The mean percentage of slow myosin molecules (65%) in the human EUS specimens investigated in this study seems very high. In six cases the proportion of slow myosin molecules ranged from 70.8% to 95.0%, which is considered to be exceptionally high in the human body, approximating that in a typical slow muscle, the soleus. It is thus clearly shown that the human EUS has the nature of typical slow muscle, maintaining a tonic contraction for hours at a time as the soleus muscle does, as previously believed without direct evidence.

The proportion of slow myosin molecules in the human EUS is considered to be very high compared with that in canine and rabbit EUS. In humans, the urethra is situated lower than the bladder for hours during standing or sitting. The human urethra seems to need more

inexhaustible continence mechanisms than those of quadrupeds. Because of bipedal walking, the pelvic floor muscles of the human being, in contrast to those of quadrupeds, are known to be extremely highly developed structures, providing support for the pelvic biscera [12]. We assume that the human EUS has become especially differentiated, in the same way as the adjacent pelvic floor musculature.

The percentages of fast and slow myosin molecules from human EUS investigated in this study varied widely between cases. This phenomenon is not seen in animals and seems to be characteristic of human EUS. It was believed earlier that the proportions of fiber types in ordinary voluntary muscle are determined only by genetic factors and do not normally change in the life time but recent research implies that fast-twitch fibers selectively fall into fatty degeneration with senility [17] and some researchers suggest the possibility that the proportions of fiber types could be changed by muscle training [18]. Our urethral specimens were from relatively old patients with bladder carcinoma. The human male EUS changes its form drastically during adolescence, because of the growth of the prostate inside it [20]. In the light of the above facts, a further study should be performed, on specimens taken from young subjects. Future studies may clarify whether such a wide variation in the proportion of fiber types in urodynamically normal human EUS as has been shown in this study is determined only by individual genetic factors or whether other factors, e.g. age and urinary habits are also involved.

It is well documented that fast- and slow-twitch muscle fibers each show the features of the ohter type after cross-reinnervation between them [4] or under continuous electric stimulation at distinct frequencies [16]. Slow-twitch fibers show the features of fast-twitch fibers following denervation [15]. It would be interesting to know whether the proportion of fiber types in the human EUS changes in different types of neurogenic bladders and/or under electric stimulation in incontinent patients.

In any case, further meticulous study on the distribution and/or proportion of fiber types in the human EUS is essential to reveal the characteristics of this least understood of muscles.

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Sohei Tokunaka, MD
Department of Urology
Asahikawa Medical College
Nishikagura 4–5
Asahikawa
078 Japan