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SKELETAL MUSCLE FIBER TYPES IN C57BL6J MICE

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ABSTRACT

Rat skeletal muscle contains up to four myosin isoforms. Analysis of single muscle fibers has identified type I, IIA, IID and IIB fibers containing type I, IIa, IId, and IIb myosin isoforms, respectively. Hybrid fibers have at least two myosin isoforms, such as myosin heavy chain (MHC) I+MHCIIa, MHCIIa+MHCIId or MHCIId+MHCIIb. These fiber types are identified as IC, IIC, IIAD, IIDA, IIDB or IIBD. The muscles of C57BL6J mice have not been extensively characterized, especially with regard to the presence and composition of hybrid fibers. The aim of this study was to examine the fiber composition of the soleus (SOL), extensor digitorum longus (EDL), tibialis anterior (TA) and gastrocnemius (GAS) muscles of C57BL6J mice using histochemical and biochemical methods. The SOL muscle contained type I fiber percentage, median ± semi amplitude, (37.42 ± 8.20%), IIA (38.62 ± 6.81%), IIAD (18.74 ± 6.95%) and IID (5.69 ± 3.09%) fibers. The EDL had type I (0.44 ± 1.27%), IC/IIC (3.05 ± 3.49%), IIA (0.46 ± 0.68%), IIAD (7.56 ± 4.51%), IID (0.46 ± 1.34%), IIDB (21.48 ± 7.33%) and IIB (66.01 ± 8.51%) fibers. The TA muscle also showed a predominance of type IIB (59.68 ± 9.95%) and IIDB (33.83 ± 15.85%) fibers followed by type IIAD (3.42 ± 4.87%), IID (2.25 ± 1.64%), IIA (1.12 ± 2.17%), I (0.17 ± 1.25%) and IC/IIC (0.008 ± 1.04%) fibers. The GAS muscle had type IIB (54.42 ± 8.11%), IIDB (19.37 ± 2.98%), IID (2.26 ± 2.24%), IIAD (12.40 ± 2.34%), IIA (5.73 ± 3.24%) and I (5.74 ± 2.55%) fibers. Thus, the SOL muscles of C57BL6J mice had predominantly type IIA fibers, unlike rats in which almost all of the fibers are type I. The EDL, TA and GAS muscles had predominantly IIB and IIDB fibers as in rats.

Key words: C57BL6J mice, muscle fiber types, myosin heavy chain

INTRODUCTION

The hindlimb muscles of adult rats contain a slow myosin isoform (MHC I) and three fast myosin isoforms (MHCIIa, IId and IIb) [19]. The IId isoform is also referred to as IIx [1,16]. Single fiber analysis has demonstrated a correlation between fibers classified by the histochemical mATPase technique and the myosin isoforms determined by electrophoresis in rabbits [22], humans [20] and rats [24]. Thus, fibers classified as type I have the type I myosin isoform (MHCI) and fibers classified as type IIA, IID and IIB have type IIa, IId and IIb isoforms.

Most muscle fibers contain only one myosin isoform and are referred to as pure fibers [23]. However, two myosin isoforms, such as MHCI+MHCIIa, MHCIIa+MHCIId or MHCIId+MHCIIb, occur in some fibers [23]. These fibers are referred to as hybrids. Those that contain MHCI and MHCIIa are classified as type IC (MHCI>MHCIIa) or type IIC (MHCIIa>MHCI). Those that contain MHCIIa and MHCIId are type IIAD (MHCIIa>MHCIId) or IIDA (MHCIId>MHCIIa), and those that contain MHCIId and MHCIIb are type IIDB (MHCIId>MHCIIb) or IIBD (MHCIIb>MHCIId) [15]. This classification suggests that there is a continuity of fiber types from the slowest to the fastest, with hybrid fibers being intermediate to pure fibers: **I**↔IC↔IIC↔**IIA**↔ IIAD↔**IID**↔IIDB↔**IIB** [15]. Although hybrid fibers are not as common as pure fibers, there is a considerable number of these fibers in adult animals [3,8,20,21]. Single fiber analysis in adult rats has shown that at least 3% of the fibers contain MHCIIa+MHCIId and 10% contain MHCIId+MHCIIb [23].

Few studies have examined the fiber composition of skeletal muscle in C57BL6J mice, especially with regard to the presence of hybrid fibers [6,7,10,12, 14,26], even though this mouse strain is used to study neuromuscular regeneration [5,11]. In this study, we examined the fiber composition of the soleus (SOL), extensor digitorum longus (EDL), tibialis anterior (TA) and gastrocnemius (GAS) muscles of C57BL6J

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mice based on the mATPase histochemical technique and on electrophoresis.

MATERIAL AND METHODS

Animals

Eight male C57BL6J mice were obtained from the Central Animal House of the State University of Campinas. The mice were housed in standard cages at a constant temperature of 25º C with free access to food and water until they were 3 months old. The mice were then anesthetized with urethane (1.5 g/kg body weight, i.p.) and the SOL, EDL, TA and GAS muscles were removed from the right hindlimb, after which the animals were euthanized by an overdose of anesthetic. The muscles were oriented in tragacanth gum and immediately frozen in isopentane cooled to -159°C in liquid nitrogen, and stored frozen at -74°C for later analysis.

Fiber typing and determination of cross-sectional area

The muscles were placed in a cryostat at -24°C and left there for 1 h before processing. The midpoint of the muscles was cut into 12 µm thick sections and the main fiber types and subtypes (I, IC/IIC, IIA, IIAD, IID, IIDB and IIB) were delineated by the mATPase technique [23] after preincubation at pH 4.35, 4.55 [4] and 10.35 [9]. The sections preincubated at pH 4.55 were mounted photographically and, in combination with those preincubated at pH 4.35 and 10.35, were examined to determine the number and percentage of each fiber type. Cross-sectional areas were measured in at least 50 fibers of each type using Image Pro Express software (Media Cybernetics®).

MHC analysis

After sections had been collected for histochemistry, 5 - 15 additional sections (12 µm) were collected for biochemical analysis. The sections were placed in 0.5 ml of a solution containing: 10% (w/v) glycerol, 5% (v/v) β-mercaptoethanol and 2.3% (w/v) sodium dodecylsulfate (SDS) in 62.5 mM Tris/HCl buffer, pH 6.8. The mixture was then shaken (1 min) and heated (10 min) to 60°C. Small portions of the extract (7-10 µl) were then run on 7-10% gradient polyacrylamide gels for 20 h at 120 V and stained with Coomassie Blue [2]. Myosin heavy chains were identified according to their apparent molecular masses compared with those of marker proteins. The gels were scanned with a Sharp scanner and the percentage of each myosin isoform was determined indirectly with Image Master software (Amersham Pharmacia).

Statistical analysis

The median and the total semi range of muscle fibers percentage, cross-sectional areas of the fibers, and myosin isoforms percentage were obtained. The results were compared using the non-parametric Friedman test with a value of $p<0.001$ indicating significance.

RESULTS

Fiber type distribution

The SOL muscle consisted of type I, IIA, IIAD and IID fibers, with a predominance of type I and IIA fibers. However, a considerable percentage of type IIAD (18.74 ± 6.95%) and IID (5.69 ± 3.09%) fibers was also observed. The EDL muscle consisted mainly of type IIB and IIDB fibers, 66.01 ± 8.51% and 21.48 ± 7.33%, respectively. A smaller number of type IIAD, IC/IIC, IIA and I fibers was also seen. The TA muscle contained predominantly type IIB and IIDB fibers, while type IIAD, IID, IIA, I and IC/IIC fibers were less abundant. Type IIB and IIDB fibers were the most numerous in GAS muscle, followed by type IIAD, I, IIA, and IID fibers (Fig. 1 and Table 1).

Cross-sectional area

Table 2 shows the cross-sectional areas of type I, IC/IIC, IIA, IIAD, IID, IIDB and IIB fibers in the muscles of C57BL6J mice. In SOL muscle, type I fibers had the largest cross-sectional area, while in EDL, TA and GAS muscles, type IIB fibers had the largest cross-sectional area (Table 2).

Myosin isoforms (MHC)

Selected rat muscles and the diaphragm of C57BL6J mice were used as a control for Myosin isoforms (MHC) analysis. The SOL muscle showed a predominance of the IIa isoform followed by MHCI and MHCIId. In EDL muscle, the IIb isoform predominated followed by isoform IIa. In TA and GAS muscles, the IIb isoform corresponded to 74.48 ± 15.15% and 84.50 ± 15.29%, respectively, followed by the IIa isoform (25.52 ± 15.15% and 17.01 ± 12.64%), respectively. In GAS muscle, the type I isoform (0.81 ± 2.34%) was also observed (Fig. 2 and Table 3).

DISCUSSION

Few studies have investigated the muscle fiber composition of C57BL6J mice [5,6,10,12-14,18,25,26]. To our knowledge, only Hämäläinen and Pette [10] have classified the types and subtypes of the muscle fibers of C57BL6J mice using the mATPase technique. However, the main objective of these investigators was not to delineate the number and percentage of muscle fibers, but rather to compare four different methods used for the mATPase reaction. Other studies have also classified the fibers of the SOL [5,12], EDL [5,12,18], TA [13] and GAS [12] muscles of C57BL6J mice, based on mATPase, NADH and SDH activities or the use of monoclonal antibodies. Different terms have been applied to those fibers, including red, intermediate or white, oxidative or glycolytic, type I, IIA and IIB, and so on.

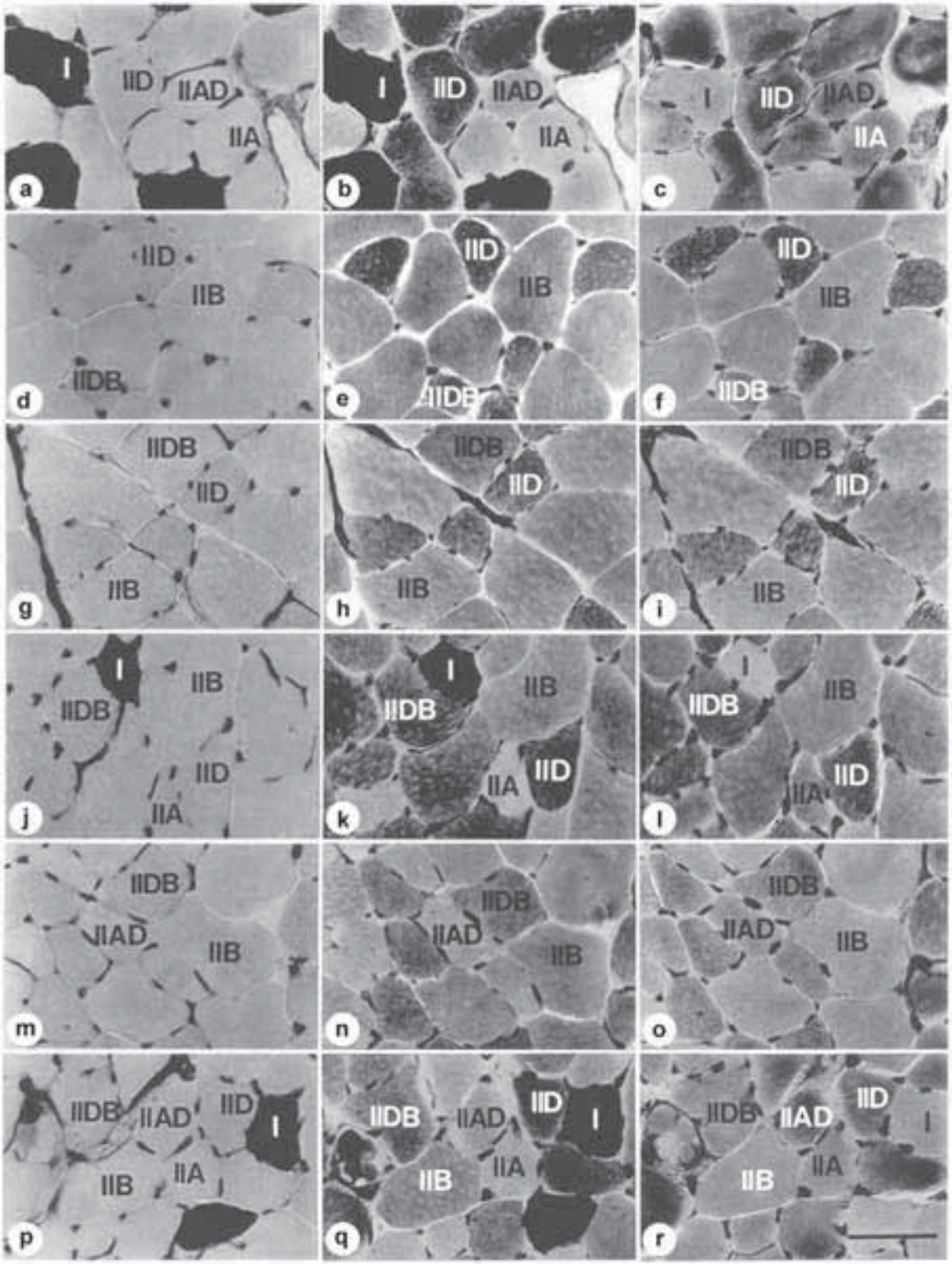

Figure 1. Serial cross-sections of the muscles: SOL (**a-c**), EDL (**d-f**), superficial area of the TA (**g-i**), deep area of the TA (**j-l**), superficial area of the GAS (**m-o**), and deep area of the GAS (**p-r**). mATPase at pH 4.35 (**a, d, g, j, m, p**), 4.55 (**b, e, h, k, n, q**) and 10.35 (**c, f, i, l, o, r**). Fiber type are indicated as I, IC, IIC, IIA, IIAD, IID, IIDB and IIB. Bar = 50 μm for all panels.

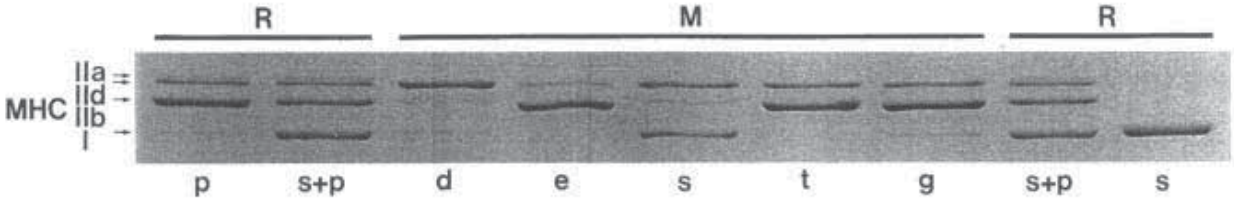

Figure 2. Electrophoretic separation of myosin heavy chain isoforms (MHCI, IIa, IId, IIb) of plantar (**p**), soleus (**s**), diaphragm (**d**), extensor digitorum longus (**e**), tibialis anterior (**t**) and gastrocnemius (**g**) muscles. **M**-mouse, **R**-rat.

Table 1. Median and total semi amplitude of the percentage of fibers in the soleus (SOL), extensor digitorum longus (EDL), tibialis anterior (TA) and gastrocnemius (GAS) muscles of male C57BL6J mice.

Fiber type	Muscle			
	SOL	EDL	TA	GAS
I	37.42 ± 8.20^{d}	0.44 ± 1.27^{a}	0.17 ± 1.25^{d}	5.74 ± 2.55^{c}
IC/IIC	0.00 ± 0.00^{a}	3.55 ± 3.49^{b}	$0.008 \pm 1{,}04^{a}$	0.00 ± 0.08^{a}
IIA	38.62 ± 6.81^{d}	0.46 ± 0.68^{a}	1.12 ± 2.17^{a}	5.73 ± 3.24^{c}
IIAD	18.74 ± 6.95^{c}	7.56 ± 4.51^{c}	3.42 ± 4.87^{b}	12.40 ± 2.34^{d}
IID	5.69 ± 3.09^{b}	0.46 ± 1.34^{a}	2.25 ± 1.64^{a}	2.26 ± 2.24^{b}
IIDB	0.00 ± 0.00^{a}	21.48 ± 7.33^{d}	33.83 ± 15.85^{c}	19.37 ± 2.98^{e}
IIB	0.00 ± 0.00^{a}	66.01 ± 8.51^{e}	59.68 ± 9.95^{d}	54.42 ± 8.11^{f}
Statistical test	47.07 (p<0.001)	42.34 (p<0.001)	43.56 (p<0.001)	46.07 (p<0.001)

The values are the median and total semi amplitude of all fibers from 8 mice. Values with different letters were significantly different (a<b<c<d<e<f). (non-parametric Friedman test, P-value 0.001).

Table 2. Median and total semi amplitude of the transversal section area (μm^2) of fibers in the soleus (SOL), extensor digitorum longus (EDL), tibialis anterior (TA) and gastrocnemius (GAS) muscles of male C57BL6J mice.

Fiber type	Muscle			
	SOL	EDL	TA	GAS
I	2005 ± 685.5^{b}	0.0 ± 255.6^{a}	710 ± 852.5^{ab}	1592 ± 419^{c}
IC/IIC	0.0 ± 0.0^{a}	535.5 ± 352^{c}	436 ± 605^{a}	0.0 ± 1125^{a}
IIA	1711 ± 467.5^{b}	206.5 ± 264.5^{b}	1205.5 ± 1262.5^{c}	1198.5 ± 203^{b}
IIAD	1594.5 ± 416.5^{b}	633 ± 633^{c}	1183 ± 1091^{c}	1105 ± 273^{b}
IID	1911 ± 1540^{b}	219.5 ± 460^{b}	951.5 ± 1363.5^{bc}	1829 ± 510.5^{d}
IIDB	0.0 ± 0.0^{a}	645.5 ± 230.5^{c}	1243.5 ± 481^{c}	1466 ± 372.5^{c}
IIB	0.0 ± 0.0^{a}	1592.5 ± 455.5^{d}	2404.5 ± 412.5^{d}	2197 ± 250^{d}
Statistical test	39.42 (p<0.001)	32.45 (p<0.001)	28.36 (p<0.001)	38.32 (p<0.001)

The values are the median and total semi amplitude of 50 fibers of each type from 8 mice. Values with different letters were significantly different (a<b<c<d<e<f). (non-parametric Friedman test, P-value 0.001).

Table 3. Median and total semi amplitude of the myosin isoforms percentage (MHC) in the soleus (SOL), extensor digitorum longus (EDL), tibialis anterior (TA) and gastrocnemius (GAS) muscles of male C57BL6J mice.

MHC	Muscle			
	SOL	EDL	TA	GAS
I	41.50 ± 12.21^{b}	0.00 ± 0.68^{a}	$0.00 \pm 0{,}0^{a}$	0.81 ± 2.34^{a}
IIa	57.56 ± 13.32^{c}	10.82 ± 6.6^{b}	25.52 ± 15.15^{b}	17.01 ± 12.64^{b}
IId	0.15 ± 2.82^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}	0.00 ± 0.0^{a}
IIb	0.00 ± 0.0^{a}	88.30 ± 6.6^{c}	74.48 ± 15.15^{c}	84.50 ± 15.29^{c}
Statistical test	22.26(p<0.001)	23.71(p<0.001)	24.00(p<0.001)	23.42(p<0,001)

The values are the median and total semi amplitude of muscle samples from 8 mice. Values with different letters were significantly different (a<b<c<d<e<f). (non-parametric Friedman test, P-value 0.001).

As shown here, in contrast to other mammals the SOL muscle of these mice showed type IID and IIAD fibers. In EDL, TA and GAS muscles, there was a predominance of type II fibers. Type I fibers were also present in GAS, TA and EDL muscles. Using monoclonal antibodies, Parry and Zardini [14] and Zardini and Parry [26] concluded that approximately 1/3 of the fibers in the TA and EDL muscles of C57BL6J mice were of the IID type. These investigators did not consider the intermediate types IIAD and IIDB. If one considers these three fiber types, then our data for the EDL and TA were similar to those reported in the literature. Thus, the SOL muscles of C57BL6J mice contained predominantly type IIA fibers, unlike rats, in which almost all of the fibers are type I. The EDL, TA and GAS muscles had predominantly type IIB and IIDB fibers, as in rats.

The cross-sectional area of type I fibers was largest in SOL muscle, whereas in EDL, TA and GAS muscles type IIB fibers had the largest cross-sectional area. Comparison of the mean fiber area of the four muscles (Table 2), showed that the largest was in TA, followed by the GAS and EDL. Parry and Wilkinson [13] reported an area of 4378 μm^2 for type IIB fibers in the superficial region of the TA muscle and an area of 3946 μm^2 in the deep region. For other fiber types, the area detected by these investigators was 1572 μm^2 on the surface and 1487 μm^2 in the deep region. Comparison of our data with those for type IIB fibers in general revealed a considerable difference. Thus, in TA muscles, the area for type IIB fibers was 2404.5 μm^2 versus 4378 μm^2 reported by Parry and Wilkinson [13]. According to Staron *et al.* [21], the cross-sectional area of muscle fibers depends on animal size and age. These same investigators reported a mean area of 2848 μm^2 for type IIB fibers in the TA muscle of Fisher 344 rats, in agreement with the present results. Pullen [17] also reported data compatible with our findings for these fiber types.

The electrophoretic analysis of muscle extracts, showed a predominance of the IIa isoform in SOL muscle followed by the type I and IId isoforms. In EDL, GAS and TA muscles, the IIb isoform predominated, followed by the IIa isoform. The type I isoform was also clearly visible in GAS muscle.

Three myosin isoforms was seen in mouse muscles (Fig. 2). In the SOL and diaphragm muscles, the isoform that migrated between isoforms IIa and I was less evident than in the EDL, TA and GAS muscles. However, the distance migrated by this myosin was practically the same in all muscles. Four isoforms were observed in rat muscles. When the isoforms with the lowest migration were compared, a clear separation of isoforms IIa and IId was seen in rat, but not in mouse, muscles. According to Zardini and Parry [26] and Parry and Zardini [14], the diaphragm muscle of C57BL6J mice does not contain the IIb isoform. Assuming that the SOL, which is a slow contracting muscle, does not have type IIB and IIDB fibers, as confirmed by histochemical analysis, then the isoform positioned between isoforms IIa and I in the SOL and diaphragm muscles of C57BL6J mice is a type IId isoform. On the other hand, since the EDL, TA and GAS muscles are predominantly fast contracting muscles with a larger number of type IIB and IID fibers, as confirmed by the mATPase reaction, they have the IId and IIb isoforms. Under the conditions used here, these isoforms migrated together and did not show adequate separation.

In conclusion, the SOL muscles of C57BL6J mice had predominantly type IIA fibers, unlike rats in which almost all of the fibers are type I. The EDL, TA and GAS muscles had predominantly IIB and IIDB fibers as in rats.

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