Individual, Age and Sex Differences in Fiber Type Composition of Slow and Fast Muscles of Adult Lewis Rats: Comparison With Other Rat Strains

P. NOVÁK[1], G. ZACHAŘOVÁ[1], T. SOUKUP[1]

[1]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

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Summary

We analyzed fiber type composition of soleus and extensor digitorum longus (EDL) muscles of 3- to 19-month-old male and female inbred Lewis rats using histochemical demonstration of mATPase activity. The rats were divided into four groups of the mean age of 3, 6, 9 and 14 months. We found that the soleus muscle of 3-month-old rats contained significantly more of fast 2A fibers and less of slow type 1 fibers compared to older rats, while no significant difference was found between female and male rats at any age group. In contrast, we found no significant difference in the EDL fiber type composition among the age groups, but we found that the EDL muscle of female rats contained significantly less 2A fibers and more 2B fibers than that of male animals. Our results thus revealed an age difference in the soleus muscle and a sex difference in the EDL muscle among postnatal Lewis rats. The number of slow type 1 fibers in the soleus muscle varied between 87 and 100 % and that of 2A fibers between 13 and 0 %. In the EDL the percentage of type 1 fibers varied between 2.6 and 8.7 %, that of 2A fibers between 12.6 and 25.8 % and that of 2B fibers between 70.4 and 81.6 %. Both muscles thus exhibited a considerable degree of variability among individual animals even in the same age group. Furthermore, a comparison of the Lewis rats with literature data of other rat strains showed that the number of fast 2A fibers in the soleus muscle of 4-month-old and older animals decreased in this order: SHR > Lister Hooded > Fisher 344 > Sprague-Dawley > Wistar > WBN/Kob > Lewis strain, being almost 20 % in the SHR and less than 2 % in the Lewis rats. In contrast, the "fastest" composition (judged according to the percentage of the fastest 2B fibers) of the EDL muscle was demonstrated by Lewis, Wistar and Fisher 344 rats (about 75 %), while Sprague-Dawley and WBN/Kob rats contained only about 50 % of 2B fibers. The percentage of slow type 1 fibers in the EDL was low in all strains (about 5 %). Our results thus show that the individual, age and sex as well as inter-strain differences in muscle fiber type composition should not be ignored when comparing results of different studies. We also demonstrated that the inbred Lewis strain appears to have more "specialized" muscle composition, as its soleus is the "slowest" and its EDL is the "fastest" among the routinely used rat strains.

Key words

Rat strains • Rat slow and fast muscles • mATPase and muscle fiber types • Fiber type composition • Inter-strain, individual, age and sex differences

Corresponding author

T. Soukup, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ-142 20 Prague, Czech Republic. E-mail: tsoukup@biomed.cas.cz, Fax: +420 2 4106 2488.

Introduction

Since Ranvier`s description of red and white muscle fibers in 1873, muscle researchers have used various classifications of fiber types, as this concept has always been important for defining the physiological properties of skeletal muscles. When Bárány (1967) demonstrated that actomyosin ATPase activity can be correlated with the speed of contraction, the histochemical demonstration of myofibrillar ATPase (mATPase) activity (Padykula and Herman 1955) became the most popular

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Fax +420 241 062 164, e-mail: physres@biomed.cas.cz, www.biomed.cas.cz/physiolres

method of fiber typing, especially after the introduction of acid and alkaline preincubations (Brooke and Kaiser 1970, Guth and Samaha 1970). While the original method allows revealing only slow type I and fast type II fibers, the acid preincubation at pH 4.5 enables the further division of fast fibers into type 2A and 2B fibers. Thus the fibers that are stained positively after acid preincubations at pH 4.3 and 4.5 of the mATPase reaction are classified as type I fibers, while the fibers that are stained positively after the alkaline preincubation at pH 10.3 and remain unstained after both acid preincubations at pH 4.3 and 4.5 are type 2A fibers and the fibers characterized by high mATPase activity after preincubation at pH 10.3 and by moderate staining after preincubation at pH 4.5 are 2B fibers. Beside these "pure" fiber types, type 1C and 2C fibers, with mixed slow and fast characteristics, stained to a variable extent after both acid and alkaline preincubations have been described (Soukup *et al.* 1979, Staron and Pette 1993, Talmadge *et al.* 1999, Smerdu and Soukup 2008, for review see Pette and Staron 1997, 2000, 2001, Stephenson 2006). Although the classification using mATPase reaction was overcome by modern division into four 1, 2A, 2X/D and 2B immunohistochemical fiber types (Soukup 2002, Zachařová *et al.* 2005, Smerdu and Soukup 2008, Soukup *et al.* 2009, for review see Hämäläinen and Pette 1993, Schiaffino and Reggiani 1996, Soukup and Jirmanová 2000, Pette and Staron 2000, 2001, Pette 2002, Vadászová *et al.* 2004, Schiaffino 2010), in the literature, there is a striking number of studies based on the mATPase classification. Despite its limitations, the mATPase reaction offers a quick, cheap and reliable assessment of fiber type composition of mammalian skeletal muscles.

The slow soleus muscle and the fast extensor digitorum longus (EDL) muscle apparently belong to the most frequently analyzed muscles, especially in small laboratory rodents. The soleus, an antigravity muscle located at the rear of the calf, is designed to sustain prolonged activity, while the EDL is a fast muscle involved in short intermittent bursts. The soleus is composed of a great majority of slow type 1 fibers and of a variable, but usually low number of 2A fibers. On the other hand, the fast EDL muscle is, according to mATPase, composed of three histochemical muscle fiber types, i.e. of a low number of slow type 1 and of variable proportions of fast 2A and 2B fibers (Soukup *et al.* 1979, 2009, for review see Pette 2001, 2002).

In the laboratory rat (*Rattus norvegicus* L.), however, the composition of both muscles can vary among different strains. Previous comparison of 4- to 6-month-old female inbred Lewis rats (Soukup *et al.* 2002) with several data collected from both sexes of other strains suggested some differences among Lewis, Wistar or Sprague-Dawley rats. Furthermore, the outcome of the fiber type analysis can be affected by differences among individual rats and by the age or sex of the analyzed animals. In our recent paper, we described fiber type composition of the soleus and EDL muscles in 4- to 17-month-old female inbred Lewis rats (Soukup *et al.* 2009), but reliable analysis of male rats is lacking.

The main goal of the present work was to analyze the contribution of individual, age, sex and strain differences to the variability of muscle fiber type composition. We have therefore i) described the composition of 3- 6-, 9- and 14-month-old age groups (range 3 to 19 months) of inbred Lewis rats of either sex, ii) compared Lewis female and male rats of the same age, iii) compared individual differences among animals in each experimental group, iv) compared our data on inbred Lewis rats, both males and females, with available literature data of other rat strains of corresponding age and sex.

Materials and Methods

Animals

Inbred Lewis rats were obtained from the breeding unit of the Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic (Accreditation No. 1020/491/A/00). The maintenance and handling of experimental animals were in accordance with the EU Council Directive (86/609EEC) and the investigation was approved by the Expert Committee of our institute. Soleus and extensor digitorum longus (EDL) muscles were excised from the right and left legs of 19 male and 79 female 3- to 19-month-old rats. The animals were divided into four age groups marked 3, 6, 9 and 14 months with mean age of 3.2±0.4, 6.0±1.3, 8.6±0.4 and 13.6±2.7 months, respectively. They were anesthetized with intraperitoneal injections of 1 ml (100 mg) of Narketan (Ketaminum ut hydrochloridum) per kg of body weight, followed by 0.5 ml (10 mg) of the myorelaxant Xylapan (Xylazinum ut hydrochloridum) per kg of body weight and sacrificed by an overdose of the anesthetic.

Myofibrillar adenosine triphosphatase (mATPase)

Muscle fiber types were determined according to the activity of mATPase (E.C.3.6.1.3) after alkaline

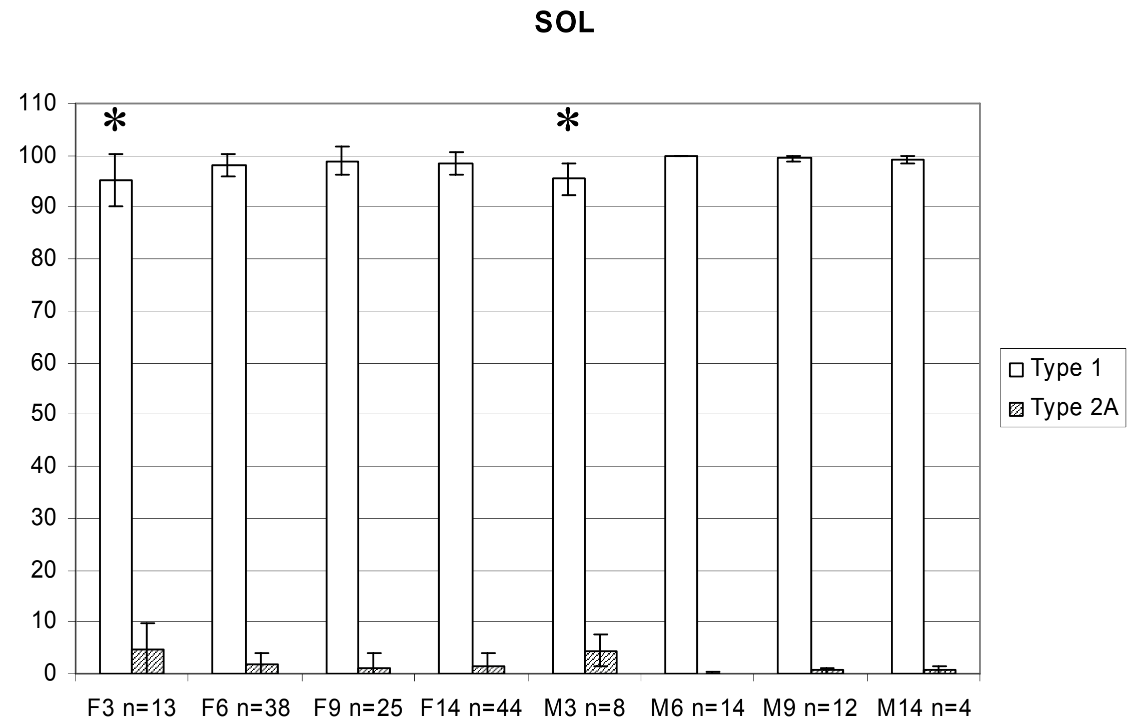

Fig. 1A. Age differences in the fiber type composition of the soleus muscle in female (F) and male (M) postnatal inbred Lewis rats in four age groups. Numerals on the x axis indicate age in months, n indicates the number of muscles analyzed. Asterisks indicate that both female and male 3-month-old rats exhibit a significantly ($p<0.05$) lower percentage of type 1 fibers when compared with the older animals. There is no significant difference in fiber type composition among 6-, 9- and 14-month-old groups of both female and male rats.

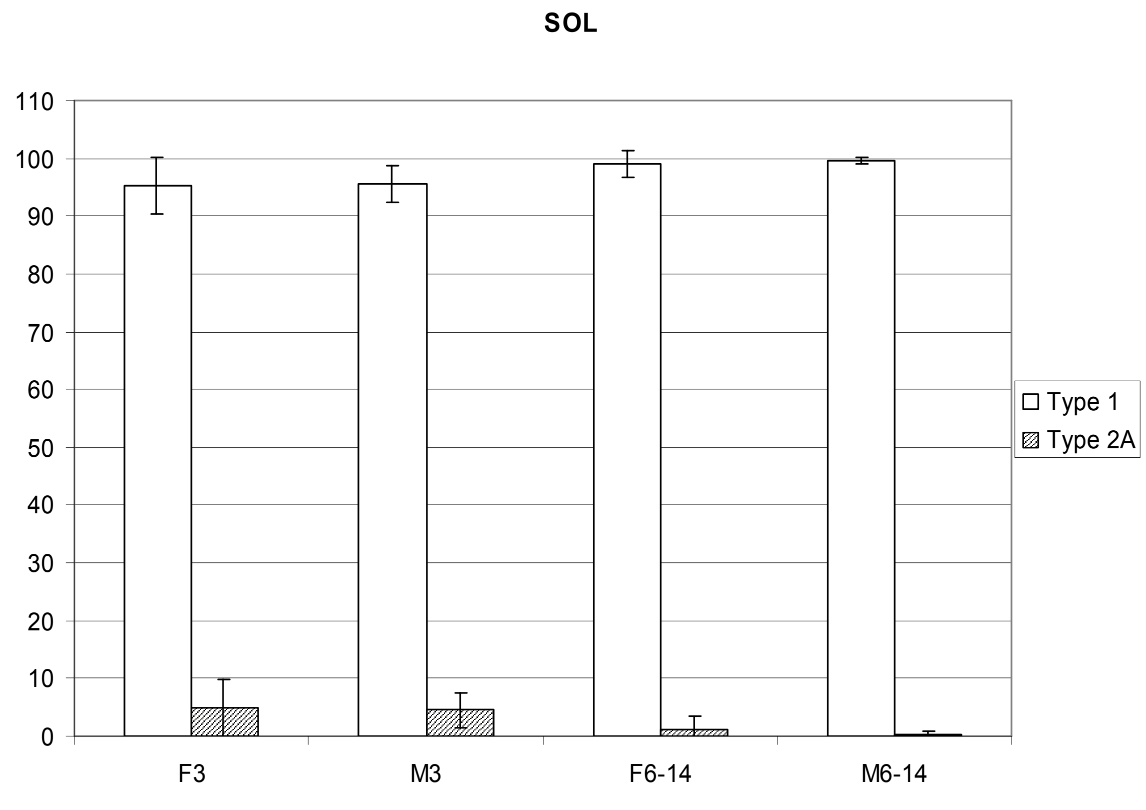

Fig. 1B. Sex differences in the fiber type composition of the soleus muscle between female (F) and male (M) inbred Lewis rats. Numerals on the x axis indicate age in months, the number of analyzed muscles is the same as in Fig. 1A. Note that there are no significant differences between female and male animals of either 3-month-old or 6- to 14-month-old groups.

(pH 10.3) and acid (pH 4.5 and 4.3) preincubations (Brooke and Kaiser 1970, Guth and Samaha 1970). Briefly, excised muscles were frozen in liquid nitrogen and cut on a Leica 3000 cryocut. Two to four 10 µm thick serial sections from the center of the muscle were collected on three glasses used for the mATPase reaction. These were followed by 10 glasses with two sections used for immunodetection of muscle fiber types using specific monoclonal antibodies against MyHC isoforms (Soukup *et al.* 2002, 2009, Smerdu and Soukup 2008). This procedure was repeated three times. The adjacent parts were used for real time detection of MyHC transcripts (Žurmanová *et al.* 2007, 2008a,b) and SDS-PAGE demonstration of MyHC isoforms (Soukup *et al.* 2002, 2009, Vadászová *et al.* 2006a,b, Vadászová-Soukup and Soukup 2007, Přenosil *et al.* 2008, Smerdu and Soukup 2008).

Quantitative morphological analysis

The numerical (N) proportions (%) of muscle fiber types, determined according to the mATPase reaction (serial sections preincubated at pH 10.3, 4.5 and 4.3), were assessed by 2-D stereological methods using the principles of an unbiased counting frame (Zachařová and Kubínová 1995). The stereological measurements were performed by the C.A.S.T. Grid System (Olympus, Albertslund, Denmark). In order to achieve realistic estimate of measured parameters, the particular arrangement of the stereological system (size of the counting frame, scanning interval) were chosen according to muscle section size and fiber composition, on the basis of efficacy analysis described in our previous papers (Zachařová and Kubínová 1995, Zachařová *et al.* 1997, 1999, 2005). The data were expressed as means ± SD, the differences were evaluated by SigmaStat program (Systat software, Germany) using the one-way analysis of variance (ANOVA) and the t-test and/or Mann-Whitney test.

Results

Fiber type composition and individual variability

By the stereological method we analyzed all muscle fibers in the cross sections through the muscle mid-belly (up to 2700 in the soleus and up to 4000 fibers in the EDL muscles).

In total, we have evaluated 160 soleus muscles, 124 from female and 36 from male rats, and the great majority of the fibers were classified as type 1 (including 1C) fibers, the rest were 2A (including 2C) (Fig. 1, Table 1). The analyzed soleus muscles i) were composed exclusively of pure type 1 fibers exhibiting high acid-stable and low alkali-stable mATPase activity (12.2 %), ii) contained practically100 % of type 1 fibers and from these, just few (1-10) fibers exhibited high dual mATPase activity thus corresponding to 1C fibers (35.1 %), iii) contained a great majority of type 1 fibers (95-99.9 %) supplemented by a small number of 2A (2C) fibers (36.6 %) or iv) contained a majority of type 1 fibers, but more than 5.5 % (up to 12.7 %) of type 2A (2C) fibers (16.1 %). The content of type 1 fibers thus varied between 87.3 to 100 % and that of 2A fibers varied from zero to 12.7 %, which demonstrates a considerable individual variability of the soleus muscle in the inbred Lewis rats.

We have analyzed 129 EDL muscles, 94 from female and 35 from male rats. All EDL muscles contained type 1, 2A and 2B fibers as determined on the basis of mATPase activity after acid preincubation at pH 4.5. The average number of 2B fibers in all examined EDL muscles greatly outnumbered 2A fibers and the number of type 1 fibers was invariably the lowest (Fig. 2, Table 2). This fiber type composition was characteristic for all EDL muscles, although a certain degree of variability occurred as well. Each fiber type contributed to the individual variability to the similar extent, but the proportions of 2A and 2B fibers varied most frequently.

We have not specifically searched for hybrid fibers (1C and 2C with the positive staining after both acid and alkali preincubations), as the stereological method does not compare individual fibers in more reactions. Analyses of fibers with acid-stable (type 1) and alkali-stable (2A, 2B) mATPase activity on serial sections showed that the average percentage of hybrid fibers with the positivity in both reactions was low both in the soleus and EDL muscles (0.6±1.5 %, range 0.0 to 3.2 %).

Age differences in fiber type composition

We have analyzed a fiber type composition of the soleus and EDL muscles in four age groups, marked 3-, 6-, 9- and 14-month-old rats. Comparison of the 3-month-old group with older groups revealed a significant difference in the type 1 and 2A composition of the soleus muscle in both sexes (Fig. 1A), but in the EDL muscle 2A and 2B proportion differed only in female rats (Fig. 2A). In the youngest group we found that about 70 % of soleus muscles contained a variable percentage of 2A fibers, but no muscle was composed purely of type 1 fibers. On the other hand, in older age groups almost 80 % of the analyzed soleus muscles in females and more than 90 % in males were solely composed of type 1 (1C) fibers. When we compared 6-, 9- and 14-month-old rats, we found no significant difference in fiber type composition either in the soleus (Fig. 1A) or EDL muscles, although 9-month-old females showed a higher percentage of type 2A and a lower percentage of 2B fibers compared to 6- and 14-month-old females in the EDL muscle (these differences were of borderline significance) (Fig. 2A).

Table 1. A comparison of our data on female and male Lewis inbred strain rats with literature data on the fiber type composition of the soleus muscle of other rat strains. The fiber type composition was determined on the basis of mATPase activity and is expressed as percentages of type 1 (including 1C) and type 2A (including 2C) fibers.

Fiber Types	Type 1 (1C)	Type 2A (2C)
LEWIS RATS		
Females, 3-4 months		
Present study	95.2±4.9	4.8±4.9
Males, 3-4 months		
Present study	95.5±3.1	4.5±3.1
ALL LEWIS RATS, 3-4 months	**95.3**	**4.7**
Females, 4-7 months		
Soukup *et al.* (2002)	96.1±2.9	3.9±2.9
Soukup *et al.* (2009) *(4.8±0.9 months)*	98.4±2.6	1.6±2.6
Zachařová *et al.* (2005)	98.8±2.2	1.2±2.2
Present study	98.2±2.2	1.8±2.2
All females, 4-7 months	***97.9***	***2.1***
Females, 7-9 months		
Soukup *et al.* (2009) *(7.4±0.8 months)*	97.3±3.0	2.7±3.0
Present study	98.9±2.7	1.1±2.7
All females, 7-9 months	***98.1***	***1.9***
Females, 9-19 months		
Soukup *et al.* (2009) *(14.1±2.3 months)*	97.8±2.7	2.2±2.7
Present study	98.4±2.2	1.6±2.2
All females, 9-19 m	***98.1***	***1.9***
Lewis females, 4-19 months	**98.0**	**2.0**
Males, 4-7 months		
Present study	99.9±0.1	0.1±0.1
Males, 7-9 months		
Present study	99.4±0.5	0.6±0.5
Males, 9-19 months		
Present study	99.3±0.7	0.7±0.7
All males, 4-19 months	***99.5***	***0.5***
ALL LEWIS RATS, 4-19 months	**98.4**	**1.6**
WISTAR RATS		
Females, 3-4 months		
Simard *et al.* (1987)	79.8±10.7	20.2±10.7

Herbison *et al.* (1973)	82.1±4.0		17.8±4.0	
Jaweed *et al.* (1975)	75.9±1.2		24.1±1.2	
Desplanches *et al.* (1987)	85.2±2.4		~14.8	
All females, 3-4 months	***80.8***		***19.2***	
Males, 3-4 months				
Yamaguchi *et al.* (1996)	85.6±7.3		8.7±5.7	(5.7±4.2)
Canon *et al.* (1995)	85±2.4	(4.2±0.7)	10.8±1.8	
Bigard *et al.* (1994)	~91		~9	
Lewis *et al.* (1994) [2]	93.1		6.9	
Sakuma *et al.* (1995)	~87		~13	
Oishi *et al.* (1996)	88.2±5.9		~11.8	
Nakano *et al.* (1995)	91.7±6.1		8.3	
Narusawa (1985)	~92.3		7.7±1.4	
All males, 3-4 months	***89.8***		***10.2***	
Other Wistar, 3-4 months				
Miyabara *et al.* (2005), n. d.	91.5±6.7	(1.4±2.6)	7.2±5.6	
Soukup *et al.* (1979), females and males	73.6	(4.7)	21.7	
WISTAR RATS, 3-4 months	**85.6**		**14.4**	
Females, adult				
Herbison *et al.* (1984)	81±5		19±5	
Aboudrar *et al.* (1993)	85.5±2.8	(7.8±2.0)	6.7±1.1	
Larsson and Yu (1997)	95 ± 5	(1±1)	3±4	(1±1)
Hall-Cragss *et al.* (1983)	89.6 (3.3)		~7.1	
Larsson and Yu (1997)	98±4	(1±1)	0±1	(1±2)
Simard *et al.* (1987)	87.0±11.7		13.0±11.7	
All females, adult	***91.5***		***8.5***	
Males, adult				
Zachařová *et al.* (1997)[1]	91.6±2 (R), 90.4±3 (L)		8.4±2 (R), 9.6±3 (L)	
Kovanen and Suominen (1987)	~89.5±7		~10.5	
Ansved (1995)	92±6	(1±1)	5±5	(2±2)
Larsson and Yu (1997)	92±6	(2±2)	4±1	(2±2)
Joumaa and Léoty (2002)	80.1±3.1		19.9±3.9	
Punkt *et al.* (1999)	80		15	(5 2B)
Midrio *et al.* (1992)	84.5		8.4	(7.0)
Chamberlain and Lewis (1989)	93.3		6.7	
Ansved (1995)	97 ± 4	(1±1)	2 ± 3	
Atrakchi *et al.* (1994) (WKY)[2]	75		25	
Li *et al.* (1996)	88.6±5.8	(3.4±1.6)	5.5±7.3	(2.4±1.0)
Larsson *et al.* (1994)	92.3±6.3	(1.6±1.8)	3.9±4.5	(2.3±2.8)
Ansved (1995)	99±1		1±1 (2C)	
Li *et al.* (1996)	99.1±1.1	(0.3±0.4)	0.1±0.2	(0.4±0.4)
Larsson *et al.* (1994)	96.3±5.7	(0.5±0.5)	1.2±2.4	(1.8±3.1)
Kovanen and Suominen (1987)	~94±5		~6	
Kovanen and Suominen (1987)	~95±5		~5	
Larsson and Yu (1997)	96±6	(1±1)	1±2	(2±3)
Thomas and Ranatunga (1993)	77±4		20±4	(3±1)
Lieber *et al.* (1986) (*inbred isogeneic*)	91.3±0.9		8.7±0.9	

All males, adult	***90.7***		***9.3***	
Wistar females and males, adult				
Soukup *et al.* (1979)	86.2		13.8	
WISTAR RATS, adult	**90.7**		**9.3**	
SPRAGUE-DAWLEY RATS				
Females, 3-4 months				
Martin and Romond (1975)	84.3±3.6		15.7±3.6	
Caiozzo *et al.* (1997)	~80		~20	
Staron *et al.* (1998)	87.4±5.7	(1.9±2.0)	5.9±2.8	(4.8±4.8)
All females, 3-4 months	***84.5***		***15.5***	
Males, 3-4 months				
Itoh *et al.* (1992)	80.8±2.5		19.2±2.2	
Eisen et al. (1975)	79.0±1.8		21.0±1.8	
Martin and Romond (1975)	83.5±1.1		16.5±1.1	
Tian and Feng (1990)	90.3±5.9		9.7±5.9	
All males, 3-4 months	***83.4***		***16.6***	
SPRAGUE-DAWLEY RATS, 3-4 months	**83.9**		**16.1**	
Females, adult				
Luginbuhl *et al.* (1984)	84.8±3.6		1.6±0.9	(13.6±2.2)
Males, adult				
Pousson *et al.* (1991)	82.8 ± 3.1		17.2 ± 2.8	
Almeida-Silveira *et al.* (1994)	85.6±5.8	(0.6±0.3)	13.8±5.6	
Ho *et al.* (1983)	83		17	
Ianuzzo *et al.*(1977)	84.0±1.4		16.0±1.4	
Ianuzzo *et al.* (1980)	83.7		16.3	
Vesely *et al.* (1999)	94±3.7		5±1.6	(1±1.1 2B)
Armstrong and Phelps (1984)	87±4		13±4	
All males, adult	***85.8***		***14.2***	
Sprague-Dawley rats, adult				
Gillespie *et al.* (1987), females and males	80		20	
Ariano *et al.* (1973), n. d.	84		16	
Lieber *et al.* (1986), n. d.	94.5		5.5	
SPRAGUE-DAWLEY RATS, adult	**85.8**		**14.2**	
FISHER 344 RATS				
Staron *et al.* (1998)	80.8±3.5	(2.1±1.6)	13.7±4.4	(3.4±2.0)
Staron *et al.* (1999)	81.9±7.4	(1.8±1.3)	9.3±5.1	(7.0±2.8)
FISHER 344 MALES, 3-4 months	**83.3**		**16.7**	
LISTER HOODED RATS				
Females, 3-4 months				
Rajinkin (1984)	~82		~18±3	
Rajinkin (1984)	~88		~12±3	
All females, 3-4 months	***85.0***		***15.0***	

Males, 3-4 months		
Rajinkin (1984)	~77	~23±3
Rajinkin (1984))	~84.5	~15.5±3
All males, 3-4 months	***80.8***	***19.3***
LISTER HOODED RATS, 3-4 months	**82.9**	**17.1**
Females, adult		
Rajinkin (1984)	~83	~17±1
Males, adult		
Rajinkin (1984)	~80	~20±5
LISTER HOODED RATS, adult	**81.5**	**18.5**
SPONTANEOUSLY HYPERTENSIVE RATS (SHR)		
Males, 3-4 months		
Lewis *et al.* (1994)	81.5±1.5	18.5±1.5
Males, adult		
Atrakchi *et al.* (1994)	81	19
SHR MALES, adult	**81**	**19**
WBN/Kob RATS		
WBN/Kob non-diabetic females, *10-24 months*		
Ozaki *et al.* (2001)[3)]	96.9	3.1
Ozaki *et al.* (2001)[3)]	97.0	3.0
All females	***97.0***	***3.1***
WBN/Kob diabetic males, *10-24 months*		
Ozaki *et al.* (2001)[3)]	95.3	4.7
Ozaki *et al.* (2001)[3)]	98.9	1.1
All males	***97.1***	***2.9***
WBN/Kob RATS, adult	**97.0**	**3.0**

[1)] Right (R) and left (L) limb, respectively; [2)] Wistar-Kyoto rats, no differences compared to normal Wistar rats were found; [3)] Classified as 2C fibers (with no type 1 fibers). Data are mean ± SD, n. d. – sex not determined.

Sex differences in fiber type composition

We did not find any significant difference in the content of type 1 and 2A fibers between male and female soleus muscles in any age group (Figs 1A,B). On the other hand, we found that the EDL muscles of the female rats contained significantly less 2A and more 2B fibers compared to the male rats, while there were no significant differences in the type 1 fiber proportion (Fig. 2B). Comparison of fiber type composition of 3-month-old and older groups of male and female rats revealed different results in soleus and EDL muscles. While a significant difference in the content of type 1 and 2A fibers between the 3-month-old and older groups occurred both in male and female soleus muscles (Fig. 1A), the EDL muscles of the 3-moth-old rats contained significantly less 2A and more 2B fibers compared to the older groups only in females, while no such tendency was observed in male rats (Figs 2A, B).

Strain differences in fiber type composition

Our data demonstrate that the soleus muscle of Lewis rats contains the highest percentage of type 1 fibers, comparable with literature data on WBN/Kob rats, but higher than the values found in Wistar and Sprague-Dawley, Fisher 344, Lister Hooded and SHR rats (Fig. 3, upper panel; Table 1). The EDL muscles in all examined strains contained a low number of type 1 fibers, varying between the lowest percentage in the Sprague-Dawley and the highest in the WBN/Kob rats (Fig. 3, lower panel; Table 2). On the other hand, the highest percentage of the

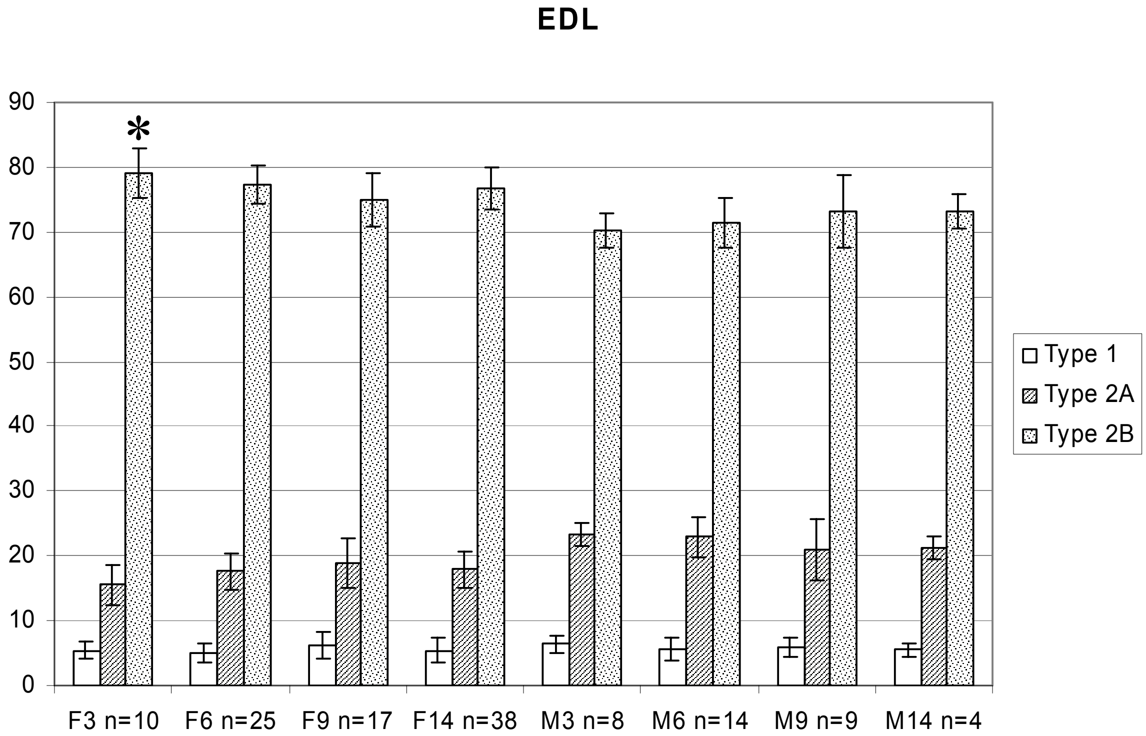

Fig. 2A. Age differences in the fiber type composition of the extensor digitorum longus (EDL) muscle in female (F) and male (M) postnatal inbred Lewis rats in four age groups. Numerals on the x axis indicate age in months, n indicates the number of muscles analyzed. Asterisk indicates that 3-month-old females, but not males, exhibit a significantly ($p<0.05$) lower percentage of 2A and a higher percentage of 2B fibers when compared with the sum of older female rats. There is no significant difference in type 1 fibers among any age groups of either sex.

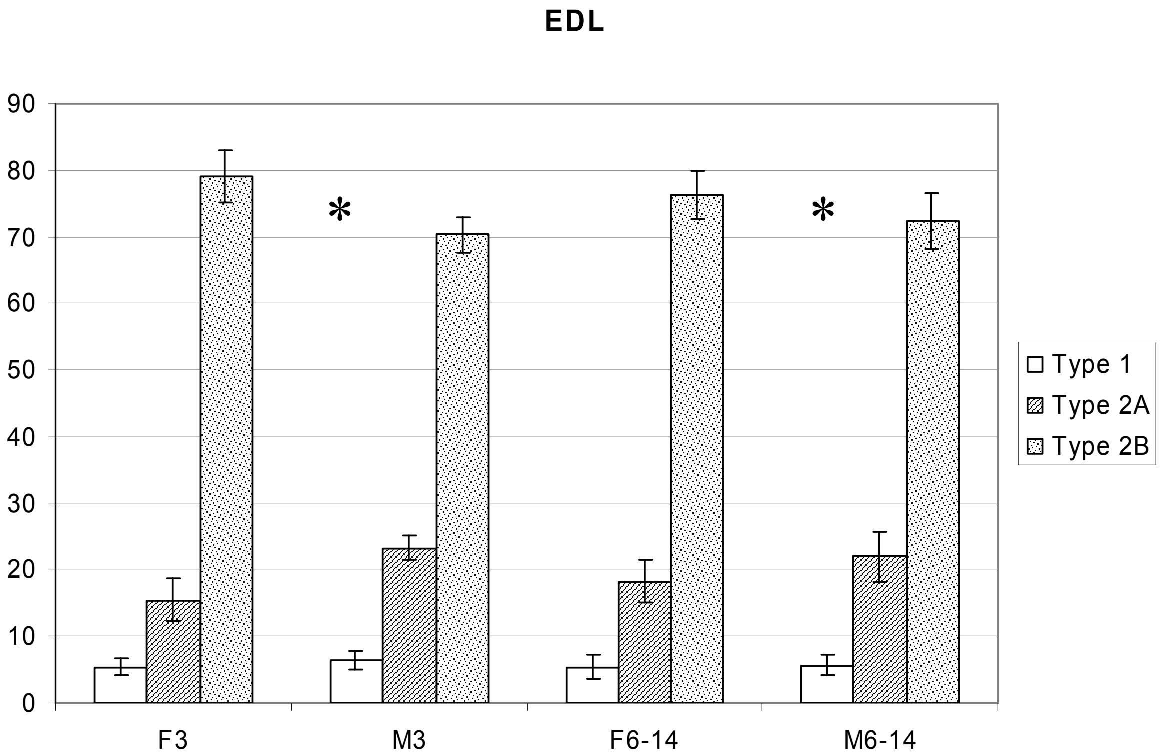

Fig. 2B. Sex differences in the fiber type composition of the extensor digitorum longus (EDL) muscle between female (F) and male (M) inbred Lewis rats. Numerals on the x axis indicate age in months, the number of analyzed muscles is the same as in Fig. 2A. Asterisks indicate that there are significant ($p<0.05$) differences in the contents of 2A and 2B fibers between female and male rats in both the 3-month-old and 6- to 14-month-old groups. The differences in the percentages of type 1 fibers are not significant.

fastest 2B fibers was exhibited by the Lewis and Wistar rats (about 75 %) and slightly lower by the Fisher 344 rats, while the Sprague-Dawley and WBN/Kob rats contained about 50 % of 2B fibers (Fig. 3, lower panel; Table 2).

Discussion

Our results on the inbred Lewis rats confirmed that i) the fiber type composition does not change after the 4th month of age, ii) the soleus of 3-month-old rats, however, contains significantly less type 1 fibers and more 2A fibers compared to older animals, iii) there is a sex difference in the proportion of 2A and 2B fiber types in the EDL muscle and iv) the fiber type composition of inbred Lewis rats differs from literature data of other routinely used rat strains.

Justification of mATPase reaction for fiber typing

As the great majority of studies analyzing fiber type composition, especially of the older ones, is based on the determination of mATPase activity, only our data dealing with this reaction were suitable for the comparison. Furthermore, in our previous study (Soukup *et al.* 2009) there was no significant difference in the percentages of type 1 and type 2A fibers in the soleus and EDL muscles based on mATPase reaction compared to immunocytochemical determination using specific monoclonal antibodies against type 1 and 2A fibers. The undisputable advantage of immunoreactions enabling separate recognition of 2X/D fibers does not bring any gain when comparing with literature data based on the division into three fiber types (1, 2A and 2B).

Fiber type composition and individual variability

Our present results for Lewis rats correspond very well with our previously published data (Soukup *et al.* 2002, 2009, Zachařová *et al.* 2005). In the soleus muscle, the percentage of 2A fibers varied between zero and 13 %, although some fibers, determined as 2A fibers, apparently bore a resemblance to hybrid 2C fibers. It is well-known that the soleus hybrid fibers exhibit physiological characteristics between slow type 1 and fast 2A fibers (for review see Pette and Staron 2001, Stephenson 2006). Although 2A fibers are capable of faster contraction, they are similarly as type 1 fibers fatigue resistant, capable to cover their metabolic requirements by the aerobic energy pathway. It can be thus hardly expected that most of the observed individual variability will have any marked effect on physiological functions of the soleus muscles. On the other hand, the EDL is a fast muscle composed in all rat strains of a low percentage of slow type 1 fibers, a medium number of fast 2A and a majority of the fastest 2B fibers. Although individual EDL muscles in Lewis rats exhibit different proportions of 2A and 2B fibers compared to mean composition, these differences (similarly as in the soleus muscles) do not suggest that they will have a significant impact on EDL muscle performance. The existence of marked fiber type differences among individual rats was already recognized previously (Hall-Craggs *et al.* 1983, Li *et al.* 1996, Soukup *et al.* 2009) and it can, however, significantly affect the fiber type percentage in studies analyzing only a low number of animals.

Our data on the Lewis rats are very reliable as they are based on the stereological evaluation of all fibers in the muscle, which is not the case in many other studies. An estimate of fiber type composition from a limited muscle sample can affect results especially in the EDL muscle, which shows considerable variation between white and red portions (Niederle and Mayr 1978). The former is composed from 2B and 2A fibers, while the latter predominantly from 2A fibers supplemented by type 1 fibers. It means that the analysis of the red portion would thus increase the percentage of 2A against 2B fibers.

Age differences in fiber type composition

There are many studies analyzing development of the soleus muscle and less of the EDL muscle during the early postnatal period, but only few of them describe fiber type composition within a longer period (Ho *et al.* 1983, Rajikin 1984, Narusawa 1985, Kovanen and Suominen 1987, Simard *et al.* 1987, Li *et al.* 1996, Wigston and English 1992, Larsson and Yu 1997). Those analyzing the soleus all describe significant increase of slow type 1 and decrease of type 2A fibers during the first two postnatal months followed by minor changes during the 3rd and 4th months of age. Our results showed that the soleus muscle of both male and female Lewis rats in the 3-month-old group still contained a lower percentage of type 1 fibers compared to older rats. The literature data on Wistar rats (Table 1) point to a similar difference in female, but not in male Wistar rats, while the data on Sprague-Dawley rats show very minor differences. Furthermore, Larsson *et al.* (1994) and Larsson and Yu (1997) reported differences between 3- to 7- and 20- to 25-month-old Wistar rats that contained about 92 and

96 % of type 1 fibers, respectively. We found a similar difference, when we selected 3- to 7- and 14- to 19-month-old female Lewis rats from our large sample, but this difference was not significant. Larsson *et al.* (1994) and Larsson and Yu (1997) reported an increase of 2A fibers on the expense of type 1 and 2B fibers in the EDL muscle of very old Wistar rats (aged 20-25 months) compared to 3- to 7-month-old ones. We have found a similar shift of 2A and 2B fibers in the EDL muscles between 3- to 7- and 14- to 19-month-old Lewis rats, but, similarly as in the soleus, this difference was not significant. The literature data on age differences of the EDL in other strains are less frequent and do not allow any suggestion. We can thus conclude that after the period of profound changes during the first three postnatal months (Kugelberg 1976, Asmussen and Soukup 1991, for review see Soukup and Jirmanová 2000, Pette and Staron 2001) the final tuning of the physiologically most proper fiber type composition of the rat soleus and EDL muscles is apparently finished by the end of the fourth month and the composition remains relatively stable throughout the whole adulthood.

Furthermore, in a recent review (Schiaffino 2010), it was shown that the age differences of muscle composition between developing and adult muscles can lead to misinterpretation of results dealing with effects of e. g. transcription factors on fiber type composition. For instance the presumed induction of fast-to-slow transformation by calcineurin (Naya *et al.* 2000) can be rather a block of the slow-to-fast switch (Schiaffino 2010) that occurs after birth in fast muscles. Similarly, the transformation block of fast-to-slow switch in the rat soleus was suggested for the effect of suspension hypokinesia performed in 3- to 4-week-old rats (Asmussen and Soukup 1991), although suspension hypokinesia is generally supposed to induce slow-to-fast transitions (e. g. Desplanches *et al.* 1987, Canon *et al.* 1995, Bigard *et al.* 1994, Caiozzo *et al.* 1997).

Table 2. A comparison of our data on female and male Lewis inbred strain rats with literature data on the fiber type composition of the EDL muscle of other rat strains. The fiber type composition was determined on the basis of mATPase activity and is expressed as percentages of type 1, type 2A and 2B fibers.

Fiber Types	**Type 1 (1C)**	**Type 2A (2C)**	**Type 2B**
LEWIS RATS			
Females, 3-4 months			
Present study	5.4±1.3	15.5±3.2	79.1±3.9
Males, 3-4 months			
Present study	6.4±1.4	23.3±1.9	70.3±2.6
LEWIS RATS , 3-4 months	**5.9**	**19.4**	**74.7**
Females, 4-7 months			
Soukup *et al*. (2002) *(4-6 months)*	5.5±1.0	18.8±1.7	75.7±2.2
Soukup *et al.* (2009) *(4.8±0.9 months)*	5.9±0.7	16.9±3.7	77.2±3.9
Present study	5.0±1.6	17.6±2.8	77.3±3.0
Zachařová *et al.* (2005) *(7.0±2.9 months)*	5.8±1.0	17.2±3.3	77.0±3.4
All females, 4-7 months	***5.6***	***17.6***	***76.8***
Females, 7-9 months			
Soukup *et al.* (2009) *(7.4±0.8 months)*	5.4±2.3	18.3±3.8	76.3±4.1
Present study	6.2±2.2	18.9±3.8	75.0±4.2
All females, 7-9 months	***5.8***	***18.6***	***75.7***
Females, 9-19 months			
Soukup *et al.* (2009) *(14.1±2.3 months)*	7.3±2.5	16.2±2.5	76.5±2.5
Present study	5.4±1.9	17.9±2.9	76.7±3.3
All females, 9-19 months	***6.4***	***17.1***	***76.6***
All Lewis females, 4-19 months	**5.8**	**17.7**	**76.5**

Males, 4-7 months			
Present study	5.6±1.7	22.9±3.1	71.4±3.7
Males, 7-9 months			
Present study	5.9±1.5	20.9±4.7	73.2±5.7
Males, 9-19 months			
Present study	5.5±1.0	21.3±1.8	73.2±2.7
All Lewis males, 4-19 months	**5.7**	**21.7**	**72.6**
LEWIS RATS, adult (4-19 months)	**5.8**	**18.8**	**75.4**
WISTAR RATS			
Wistar, females and males, 3-4 months			
Soukup *et al.* (1979)	4.5 (2.3)	27.8	65.4
Males, 3-4 months			
Bigard *et al.* (1994)	~4	~20	~76
WISTAR RATS, 3-4 months	**5.4**	**23.9**	**70.7**
Females, adult			
Larsson and Yu (1997) *(4-7 months)*	4±1	14±4	79±6
Larsson and Yu (1997) *(21-25 months)*	3±1	10±7	87±6
All females, adult	***3.5***	***12.0***	***83.0***
Males, adult			
Larsson *et al.* (1994) *(3-6 months)*	3.4±1.1	18.7±4.7	76.1±4.4
Green *et al.* (1984)	7.7, 3.1	22.1, 16.2	70.2, 80.7
Larsson and Yu (1997) *(4-7 months)*	4±1	21±6	75±6
Larsson *et al.* (1994) *(20-24 months)*	3.3±0.8	23.3±6.4	72±6.1
Larsson and Yu (1997) *(21-25 months)*	3±1	23±6	72±6
All males, adult	***4.1***	***20.7***	***74.3***
WISTAR RATS, adult	**3.9**	**18.5**	**76.5**
SPRAGUE-DAWLEY RATS			
Males, 3-4 months			
Tian and Feng (1990)	3.0±1.9	97.0±1.9 (type II)	
Males, adult			
Vesely *et al.* (1999)	7±2.0	45±2.4	48±1.8
Armstrong and Phelps (1984)	2±1	42±7	56±8
Ariano *et al.* (1973), n.d.	3	59	38
Egginton (1990), n.d.	3	36.2	60.8
SPRAGUE-DAWLEY RATS, adult	**3.8**	**45.6**	**50.7**
WBN/Kob RATS			
Non-diabetic females, 10-24 months			
Ozaki *et al.* (2001)	8.3	48.9	42.8
Ozaki *et al.* (2001)	8.2	50.5	41.3

All females	**8.3**	**49.7**	**42.1**
Diabetic males, 10-24 months			
Ozaki *et al.* (2001)	8.2	50.0	41.9
Ozaki *et al.* (2001)	7.5	39.5	53.0
All males	**7.9**	**44.8**	**47.5**
WBN/Kob RATS, adult	**8.1**	**47.2**	**44.8**
FISHER 344 RATS			
Males, 3-4 months			
Staron *et al.* (1999)	4.0±1.6 (0.8±0.6)	15.5±2.8 (0.6±0.6) 7.3±3.4 (IIAD)	29.9±4.9 36.5±4.6 (IID) 5.4±2.9 (IIDB)
Males, adult			
Kraemer *et al.* (2000)	4.4±1.4 (0.9±0.7)	16.5±2.0 (0.9±1.0) 7.7±1.5 (IIAD)	26.4±2.7 36.9±2.0 (IID) 6.3±1.5 (IIDB)
FISHER 344 RATS, adult	**5.3**	**25.1**	**69.6**

Data are mean ± SD, n. d. – sex not determined.

Sex differences in fiber type composition

Our data did not reveal any significant sex difference in the composition of the soleus muscle, although adult males contained higher percentages of type 1 (and lower percentages of 2A) fibers than females as more male than female soleus muscles were solely composed of slow type 1 and 1C fibers. It also appeared that soleus muscles in males achieved their "slow" composition earlier than in female Lewis rats. We speculate that these differences can be correlated with different growth rates of female and male rats as evident already four weeks after birth (http://www.harlan.com/research_models_and_services). The literature data on adult Wistar, Sprague-Dawley and WBN/Kob rats show no sex difference between female and male soleus muscles, with one exception, i. e. Wistar young females contained less type 1 fibers than the male rats of the same age, which can be again related to faster growth in males (http://www.harlan.com/research_models_and_services) (Table 2). Although the most evident growth differences between females and males are reported for the Sprague-Dawley rats (http://www.harlan.com/research_ models_ and_services), the collected literature data do not show any difference in the soleus fiber type composition either in young or older animals (Table 1). On the other hand, we found a significant difference in the EDL muscle between female and male inbred Lewis rats, as females contained more of faster 2B and less of 2A fibers compared to male EDL muscles (Fig. 2B). The latter difference also appeared from the comparison of literature data on Wistar rats (Table 2). No sex difference was detected in the soleus muscle of 2.5-months-old CFHB-Wistar rats (Pullen 1977) and between WBN/Kob non diabetic female and diabetic male rats (Ozaki *et al.* 2001). On the other hand, a consistently higher proportion of 2A fibers was found in the soleus of 4- to 20-week-old Lister Hooded male rats compared to female rats (Rajikin 1984). The same author speculated that this difference (that was the highest at 8 and 12 weeks of age, i.e. in puberty) can be caused by differences in the level of circulating testosterone. The sex differences observed in limb muscles are quite small which is in contrast with the sexually dimorphic muscles, like guinea pig temporalis or rat levator ani muscles (d`Albis *et al.* 1991).

Strain differences in fiber type composition

Comparison of fiber type composition of different rat strains demonstrates that the soleus muscle of Lewis rats is the "slowest", as it exhibits the highest percentage of type 1 fibers, followed by WBN/Kob, Wistar and Sprague-Dawley, Fisher 344, Lister Hooded and SHR rats (Table 1, Fig. 3). Furthermore, the inbred

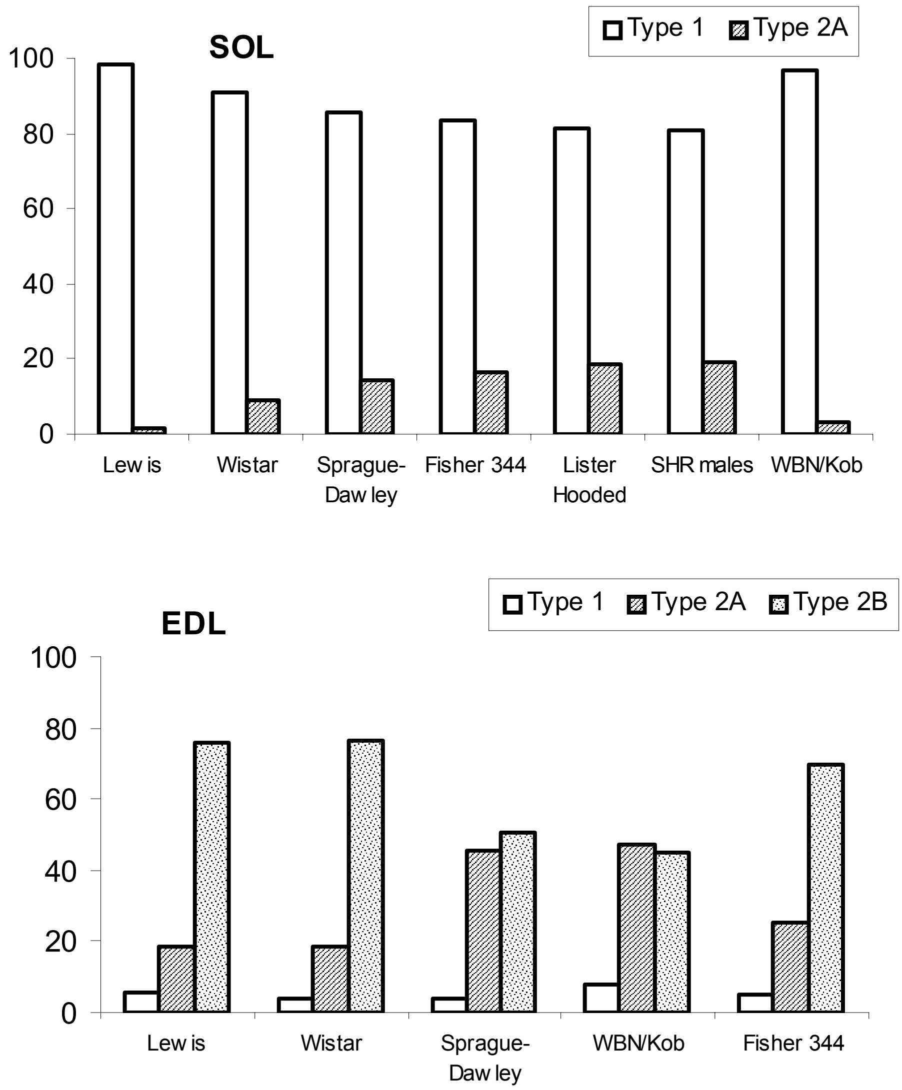

Fig. 3. Mean fiber type composition of the soleus (SOL, upper panel) and extensor digitorum longus (EDL, lower panel) muscles of 4-month-old and older rats as summarized from the literature data on different rat strains (for further details see Tables 1 and 2).

Lewis rats attain the very high percentage of type 1 fibers in the soleus muscle earlier than the other strains. It can be related to the higher natural levels of serum thyroxine (http://www.harlan.com/research_models_and_services). This fact was demonstrated experimentally, as hyperthyroid rats achieved adult soleus composition earlier than euthyroid and hypothyroid rats (Vadászová-Soukup and Soukup 2007). In the EDL muscle, the highest percentage of the fastest 2B fibers (and the lowest of 2A fibers) was exhibited by Lewis, Wistar and Fisher rats, while Sprague-Dawley and WBN/Kob contained an almost equal percentage of 2B and 2A fibers (Table 2, Fig. 3).

It was shown that the soleus muscle of SHR rats contains a three times greater proportion of fast fibers and its twitch contraction and relaxation time is 12-15 % faster compared to normotensive WKY rats (Lewis *et al.* 1994). This means that the increase of about 14 % of fast 2A fibers leads to a similar percentage change of physiological parameters. Corresponding or even higher differences in contraction and relaxation time can be expected e. g. between SHR and Lewis rats as the percentage of the type 1 fibers in soleus muscles ranges from about 80 % in the SHR to almost 99 % in the Lewis rats. Similarly, the difference in the content of type 2B in the EDL between Sprague-Dawley or WBN/Kob (about 50 %) and Lewis or Wistar rats (about 75 %) seems to be high enough to have physiological consequences. Our results show that regarding soleus fiber type composition, Lewis and WBN/Kob rats form a group of "very slow" strains, while Sprague-Dawley, Fisher 344, Lister Hooded and SHR rats correspond to the "relatively faster" strains, with Wistar rats in between these two strain groups. Regarding the EDL, however, the Lewis and Wistar rats form the "fast" group, while the Sprague-Dawley and WBN/Kob rats represent the "relatively slower" strains, the Fisher 344 rats being in between these groups. It seems that the muscle fiber type composition is specific for the given strain regardless of it being inbred or outbred. Although we followed only the soleus and EDL muscles, it can be supposed that similar strain differences are present in other or even in all skeletal muscles. The strain differences thus must not be ignored in comparative studies, when a comparison of physiological results of different strains is made.

Conclusions

Our results revealed substantial individual variability in muscle fiber type composition both in the soleus and EDL muscles, age differences in the soleus and sex differences in the EDL muscles. A comparison of the Lewis and other rat strains revealed obvious inter-strain differences, which demonstrate that for comparative studies, the inter-strain differences must be seriously considered. The results also show that the inbred Lewis rats appear to be the most "specialized" in respect to skeletal muscle composition, as their soleus is the slowest and their EDL is the fastest among compared rat strains.

Conflict of Interest

There is no conflict of interest.

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