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

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Running title: fiber type variability of rat slow and fast muscles

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SUMMARY

We analyzed fiber type composition of the soleus and extensor digitorum longus (EDL) muscles of 3- to 19-month-old male and female inbred Lewis strain rats determined by 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 strain 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. They also show 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

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. After the paper by Bárány (1967) demonstrating 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 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 (e. g. Soukup *et al.* 1979, Staron and Pette 1993, Talmadge *et al.* 1999, Smerdu and Soukup 2008, for review see e.g. 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 (e.g. Soukup 2002, Zacharova *et al.* 2005, Smerdu and Soukup 2008, Soukup *et al.* 2009, for review see e.g. Hämäläinen and Pette 1993, Schiaffino and Reggiani 1996, Soukup and Jirmanová 2000, Pette and Staron 2000, 2001, Pette 2002, Vadászová *et al.* 2004), in the

literature, there is a striking number of studies based on the mATPase classification. Despite its limitations, the mATPase reaction namely 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 (e. g. Soukup *et al.* 1979, 2009, for review see e.g. 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 strain rats (Soukup *et al.* 2002) with several data collected from both sexes of other strains suggested some differences between 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 strain 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 male and female, with available literature data of other rat strains of corresponding age and sex.

Materials and Methods

Animals. Inbred Lewis strain rats were obtained from the authorized laboratory rat-breeding unit of the Institute of Physiology, Academy of Sciences of the Czech Republic, v. v. i., 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 the Institute of Physiology, Academy of Sciences of the Czech Republic, v. v. i., Prague, Czech Republic. 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 Narkamon 5 % (Spofa, Prague, Czech Republic, 100 mg/kg of body weight) and Rometar 2 % (Spofa, Prague, Czech Republic, 10 mg/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 (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 and point

counting (Zacharova 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 concrete arrangement of the stereological system (number of points, 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 (Zacharova and Kubinova 1995, Zacharova *et al.* 1997, 1999, 2005). The data were expressed as means ± SD, the differences between groups were tested by one way analysis of variance (ANOVA) and the significance ($p<0.05$) was evaluated by SigmaStat program (Systat software, Germany) using 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 fibers were classified as type 1 (including 1C) fibers, the rest were 2A (including 2C) (Fig. 1, Table 1). Analyzed soleus muscles were composed i) 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 strain 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 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 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 the F9 female group showed a higher percentage of type 2A and a lower percentage of 2B fibers compared to F6 and F14 groups in the EDL muscle (these differences are at the border of significance) (Fig. 2A).

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 (Fig. 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 the 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 Wistar and Sprague-Dawley, Fisher 344, Lister Hooded and SHR rats (Fig. 3A, Table 1). The EDL muscles in all examined strains contained a low number of type 1 fibers, varying between the lowest percentage in Sprague-Dawley and the highest in WBN/Kob rats (Fig. 3B, Table 2). On the other hand, the highest percentage of the fastest 2B fibers was exhibited by Lewis and Wistar rats (about 75 %) and slightly lower by Fisher 344 rats, while Sprague-Dawley and WBN/Kob rats contained less than 50 % of 2B fibers (Fig. 3B, Table 2).

Discussion

Our results on the inbred Lewis strain 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 strain 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) we found no significant difference in the percentage 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, Zacharova *et al.* 2005). In the soleus muscle, the percentage of 2A fibers varied between zero and up to about 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 strain 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 (e.g. 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 and less of the EDL muscle during the early postnatal period, but only few of them describe fiber type composition within a longer period (e. g. 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. Our results showed that both male and female Lewis rats in the 3-month-old group still contained in the soleus muscle 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 difference 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 (20 to 25 months) compared to 3- to 7-month-old ones. We have found a similar shift of 2A and 2B 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.

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 related to faster growth in males (http://www.harlan.com/research_models_and_services) (cf. Table 2). Although the most evident growth differences between females and males are reported for 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 (cf. 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 speculates that this difference (that was the highest at 8 and 12 weeks of age, i. e. the time of puberty) can be caused by differences in the level of circulating testosterone. The sex differences observed in limb muscles are, however, 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. 3A). Furthermore, the inbred 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 eu- 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. 3B).

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 SHR to almost 99 % in Lewis rats. Similarly, the 25 percentage-point 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 correspond to the "relatively faster" strains, with Wistar rats in between these two groups. Regarding the EDL, however, Lewis and Wistar rats form the "fast" group, while Sprague-Dawley and WBN/Kob represent the "relatively slower" strains, the Fisher 344 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, as well when a comparison of physiological results of different strains is necessary.

Conclusion. 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 difference, which demonstrates that for comparative studies, the inter-strain differences must be seriously considered. The results also show that the inbred Lewis strain 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.

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Text to tables

Table 1. Literature data of type 1 (including 1C) and type 2A (including 2C) fibers determined on the basis of mATPase activity and expressed as percentages of fiber types (mean ± SD or SEM) in the soleus muscle of female and male rats of different age from several rat strains (m=age in months, n. d.=sex not determined).

Table 2. Literature data of type 1 (including 1C), 2A (including 2C) and 2B fibers determined on the basis of mATPase activity and expressed as percentages of fiber types (mean ± SD or SEM) in the EDL muscle of female and male rats of different age from several rat strains (m=age in months, n. d.=sex not determined).

Text to figures

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

Fig. 2A. Fiber type composition of the extensor digitorum longus (EDL) muscle in female (F) and male (M) postnatal inbred Lewis strain rats in four age groups. Numerals on the x axis indicate age in months, n indicates the number of muscles analyzed. Note that 3-month-old females, but not males, exhibit a significantly lower percentage of 2A and a higher percentage of 2B fibers when compared with the sum of older female or male rats (* indicates a significant difference, $p < 0.05$). Note also that 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 strain rats. Numerals on the x axis indicate age in months, the number of analyzed muscles is the same as in Fig. 2A. Note that there is a significant difference between female and male rats between 3-month and 6- to 14-month groups of female and male animals in the contents of 2A and 2B fibers (* indicates a significant difference between female and male animals, $p < 0.05$). The differences in the percentages of type 1 fibers are not significant.

Fig. 3A,B. Mean fiber type composition of the soleus (SOL, A) and extensor digitorum longus (EDL, B) 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).

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Table 1

FIBER TYPES	**Type 1 (1C)**	**Type 2A (2C)**
INBRED LEWIS RATS		
Females, 3-<4 m		
Present study	95.2±4.9	4.8±4.9
Males, 3-<4 m		
Present study	95.5±3.1	4.5±3.1
LEWIS RATS, 3-<4m	**95.3±0.2**	**4.7±0.2**
Females, 4-7 m		
Soukup *et al.* 2002	96.1±2.9	3.9±2.9
Soukup *et al.* 2009 *(4.8±0.9 m)*	98.4±2.6	1.6±2.6
Zacharova *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 m	**97.9±1.2**	**2.1±1.2**
Females, >7-9 m		
Soukup *et al.* 2009 *(7.4±0.8 m)*	97.3±3.0	2.7±3.9
Present study	98.9±2.7	1.1±2.7
All females, >7-9 m	**98.1±1.1**	**1.9±1.1**
Females, >9-19 m		
Soukup *et al.* 2009 *(14.1±2.3 m)*	97.8±2.7	2.2±2.8
Present study	98.4±2.2	1.6±2.2
All females, 9-19 m	**98.1±0.4**	**1.9±0.4**
Inbred Lewis females, 4-19 m	**98.0±0.9**	**2.0±0.9**
Males, 4-7 m		
Present study	99.9±0.1	0.1±0.1
Males, >7-9 m		
Present study	99.4±0.5	0.6±0.5
Males, >9-19 m		
Present study	99.3±0.7	0.7±0.7
Inbred Lewis males, 4-19 m	**99.5±0.3**	**0.5±0.3**
LEWIS RATS, adult (4-19 months)	**98.4±1.1**	**1.6±1.1**
WISTAR RATS		
Females, 3-<4 m		
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 m	**80.8±3.9**	**19.2±3.9**
Males, 3-<4 m		
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 m	**89.8±2.7**	**10.2±2.7**
Other Wistar, 3 – <4 m		
Miyabara *et al.* 2005, n. d.	91.5±6.7 (1.4±2.6)	7.2±5.6

Soukup *et al.* 1979, F+M	73.6 (4.7)	21.7
WISTAR RATS, 3-<4m	**86.6±5.7**	**13.4±5.7**
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±6.5**	**8.5±6.5**
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±7.2**	**9.3±7.2**
Wistar F+M, adult		
Soukup *et al.* 1979	86.2	13.8
WISTAR RATS, adult	**90.7±6.8**	**9.3±6.8**
SPRAGUE-DAWLEY RATS		
Females, 3-<4 m		
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 m	**84.5±4.7**	**15.5±4.7**
Males, 3-<4 m		
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 m	**83.4±5.0**	**16.6±5.0**
SPRAGUE-DAWLEY RATS, 3-<4 m	**83.9±4.5**	**16.1±4.5**
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±3.9**	**14.2±3.9**
Sprague-Dawley F+M and n. d., adult		
Gillespie *et al.* 1987, F+M	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±4.5**	**14.2±4.5**
FISHER 344 MALES, 3-<4 m		
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 m	**83.3±0.6**	**16.7±0.6**
LISTER HOODED RATS		
Females, 3-<4 m		
Rajinkin 1984	~82	~18±3
Rajinkin 1984	~88	~12±3
All females, 3-<4 m	**85.0±4.2**	**15.0±4.2**
Males, 3-<4 m		
Rajinkin 1984	~77	~23±3
Rajinkin 1984	~84.5	~15.5±3
All males, 3-<4 m	**80.8±5.3**	**19.3±5.3**
LISTER HOODED RATS, 3-<4 m	**82.9±4.6**	**17.1±4.6**
Females, adult		
Rajinkin 1984	~83	~17±1
Males, adult		
Rajinkin 1984	~80	~20±5
LISTER HOODED RATS, adult	**81.5±2.1**	**18.5±2.1**
SHR MALES		
Males, 3-<4 m		
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 nondiabetic females, *10-24 m*		
Ozaki *et al.* 2001[3)]	96.9	3.1
Ozaki *et al.* 2001[3)]	97.0	3.0
All females	**97.0±0.1**	**3.1±0.1**
WBN/Kob diabetic males, *10-24 m*		
Ozaki *et al.* 2001[3)]	95.3	4.7
Ozaki *et al.* 2001[3)]	98.9	1.1
All males	**97.1±2.5**	**2.9±2.5**

WBN/Kob RATS, adult	**97.0±1.5**	**3.0±1.5**

[1)] Right (R) and left (L) limb, respectively
[2)] Wistar-Kyoto strain, no differences compared to normal Wistar strain were found
[3)] Classified as 2C fibers (with no type 1 fibers)

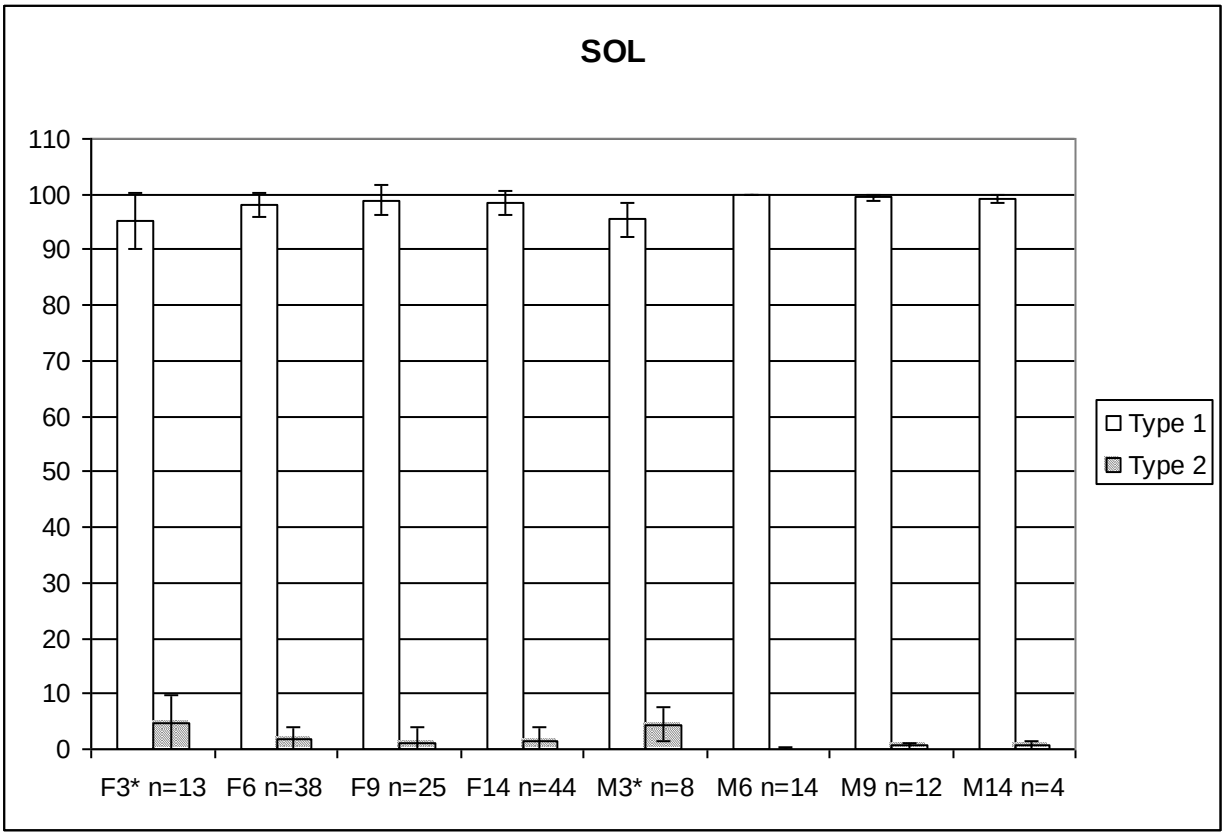
Table 2

FIBER TYPES	Type 1 (1C)	Type 2A (2C)	Type 2B
INBRED LEWIS RATS			
Females, 3-<4 m			
Present study	5.4±1.3	15.5±3.2	79.1±3.9
Males, 3-<4 m			
Present study	6.4±1.4	23.3±1.9	70.3±2.6
LEWIS RATS , 3-<4 m	**5.9±0.7**	**19.4±5.5**	**74.7±6.2**
Females, 4-7 m			
Soukup *et al.* 2002 *(4-6 m)*	5.5±1.0	18.8±1.7	75.7±2.2
Soukup *et al.* 2009 *(4.8±0.9 m)*	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
Zacharova *et al.* 2005 *(7.0±2.9 m)*	5.8±1.0	17.2±3.3	77.0±3.4
All females, 4-7 m	**5.6±0.4**	**17.6±0.8**	**76.8±0.7**
Females, >7-9 m			
Soukup *et al.* 2009 *(7.4±0.8 m)*	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 m	**5.8±0.6**	**18.6±0.4**	**75.7±0.9**
Females, >9-19 m			
Soukup *et al.* 2009 *(14.1±2.3 m)*	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 m	**6.4±1.3**	**17.1±1.2**	**76.6±0.1**
Inbred Lewis females, 4-19 m	**5.8±0.7**	**17.7±0.9**	**76.5±0.8**
Males, 4-7 m			
Present study	5.6±1.7	22.9±3.1	71.4±3.7
Males, >7-9 m			
Present study	5.9±1.5	20.9±4.7	73.2±5.7
Males, >9-19 m			
Present study	5.5±1.0	21.3±1.8	73.2±2.7
Inbred Lewis males, 4-19 m	**5.7±0.2**	**21.7±1.1**	**72.6±1.0**
LEWIS RATS, adult (4-19 months)	**5.8±0.6**	**18.8±2.1**	**75.4±2.0**
WISTAR RATS			
Wistar, F+M, 3-<4 m			
Soukup *et al.* 1979	4.5 (2.3)	27.8	65.4
Males, 3-<4 m			
Bigard *et al.* 1994	~4	~20	~76
WISTAR RATS, 3-<4	**5.4±2.0**	**23.9±5.5**	**70.7±7.5**
Females, adult			
Larsson and Yu 1997 *(4-7 m)*	4±1	14±4	79±6
Larsson and Yu 1997 *(21-25 m)*	3±1	10±7	87±6
All females, adult	**3.5±0.7**	**12.0±2.8**	**83.0±5.7**
Males, adult			
Larsson *et al.* 1994 *(3-6m)*	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 m)*	4±1	21±6	75±6
Larsson *et al.* 1994 *(20-24 m)*	3.3±0.8	23.3±6.4	72.0±6.1
Larsson and Yu 1997 *(21-25 m)*	3±1	23±6	72±6
All males, adult	**4.1±1.8**	**20.7±2.8**	**74.3±3.8**
WISTAR RATS, adult	**3.9±1.6**	**18.5±4.8**	**76.5±5.6**

SPRAGUE-DAWLEY RATS			
Males, 3-<4 m			
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±2.2**	**45.6±9.7**	**50.7±10.0**
WBN/Kob RATS			
Nondiabetic females, *10-24 m*			
Ozaki *et al.* 2001	8.3	48.9	42.8
Ozaki *et al.* 2001	8.2	50.5	41.3
All females	**8.3±0.1**	**49.7±1.1**	**42.1±1.1**
Diabetic males, *10-24 m*			
Ozaki *et al.* 2001	8.2	50.0	41.9
Ozaki *et al.* 2001	7.5	39.5	53.0
All males	**7.9±0.5**	**44.8±7.4**	**47.5±7.8**
WBN/Kob RATS, adult	**8.1±0.4**	**47.2±5.2**	**44.8±5.5**
FISHER 344 RATS			
Males, 3-<4 m			
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**

Fig. 1

1A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100
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80
70
60
50
40
30
20
10
0
F3* n=13
F6 n=38
F9 n=25
F14 n=44
M3* n=8
M6 n=14
M9 n=12
M14 n=4
Type 1
Type 2

1B

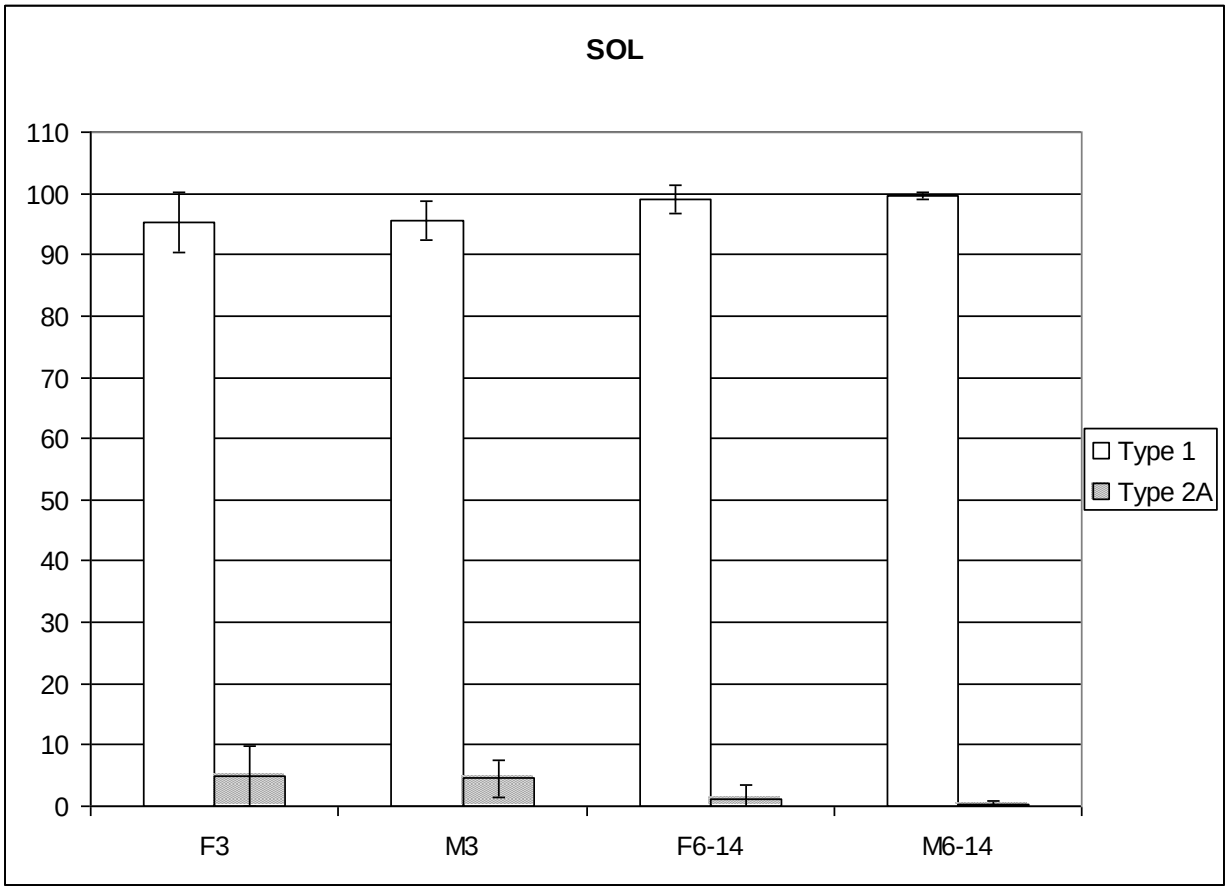
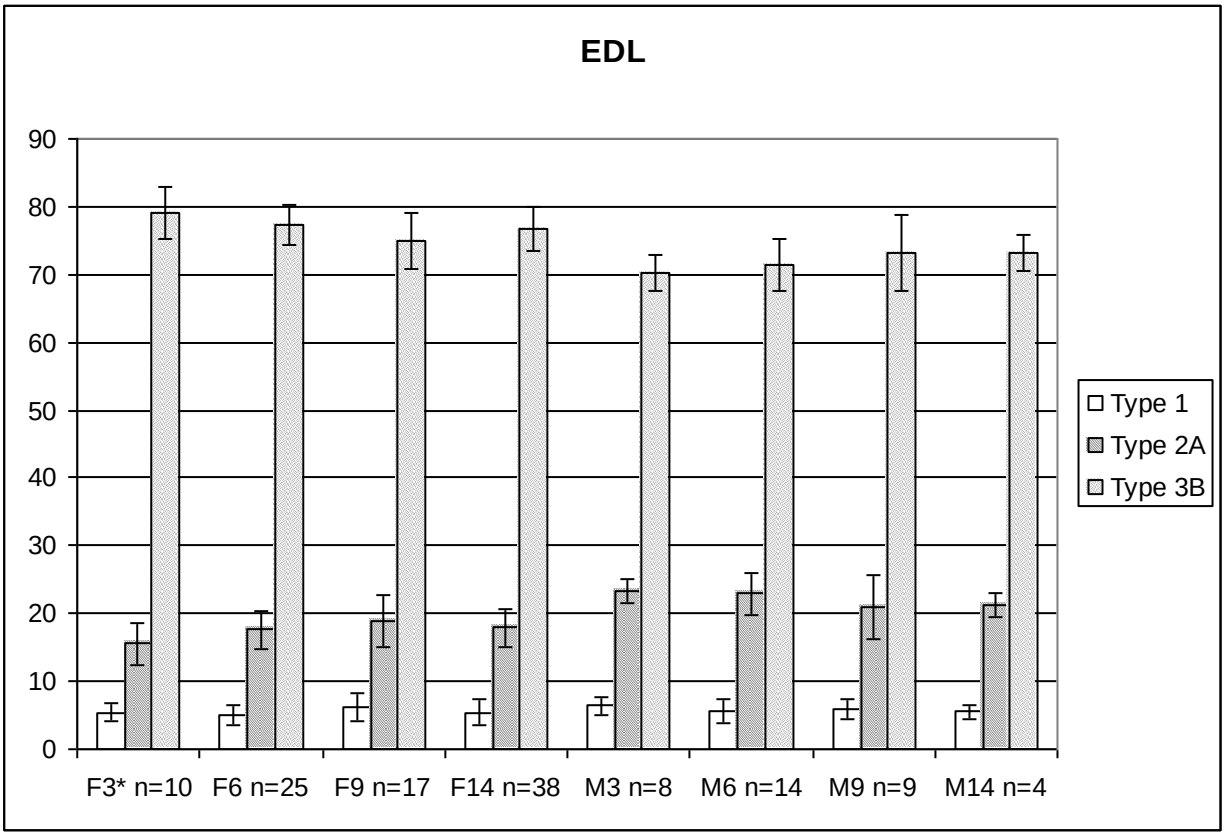

SOL
110
100
90
80
70
60
50
40
30
20
10
0
F3
M3
F6-14
M6-14
Type 1
Type 2A

Fig. 2
2A


EDL
90
80
70
60
50
40
30
20
10
0
F3* n=10
F6 n=25
F9 n=17
F14 n=38
M3 n=8
M6 n=14
M9 n=9
M14 n=4
Type 1
Type 2A
Type 3B

2B

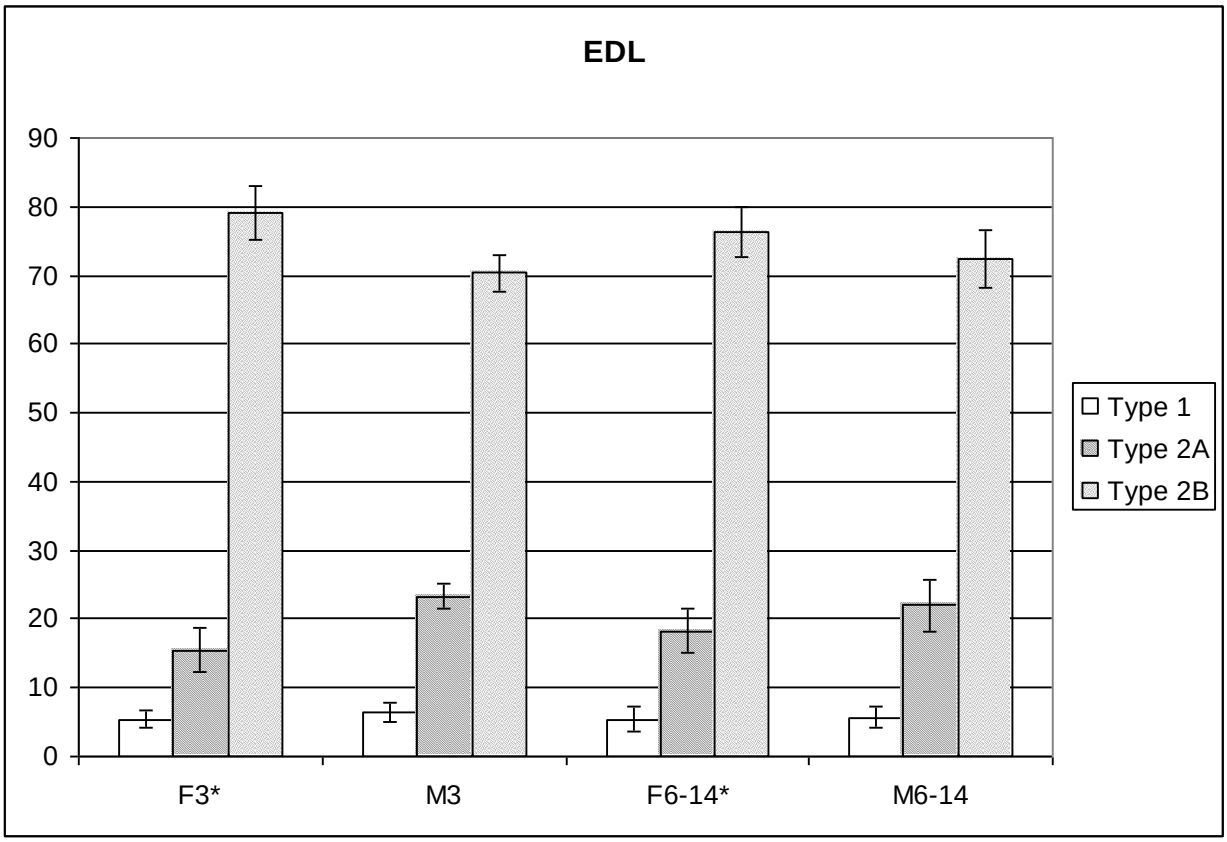
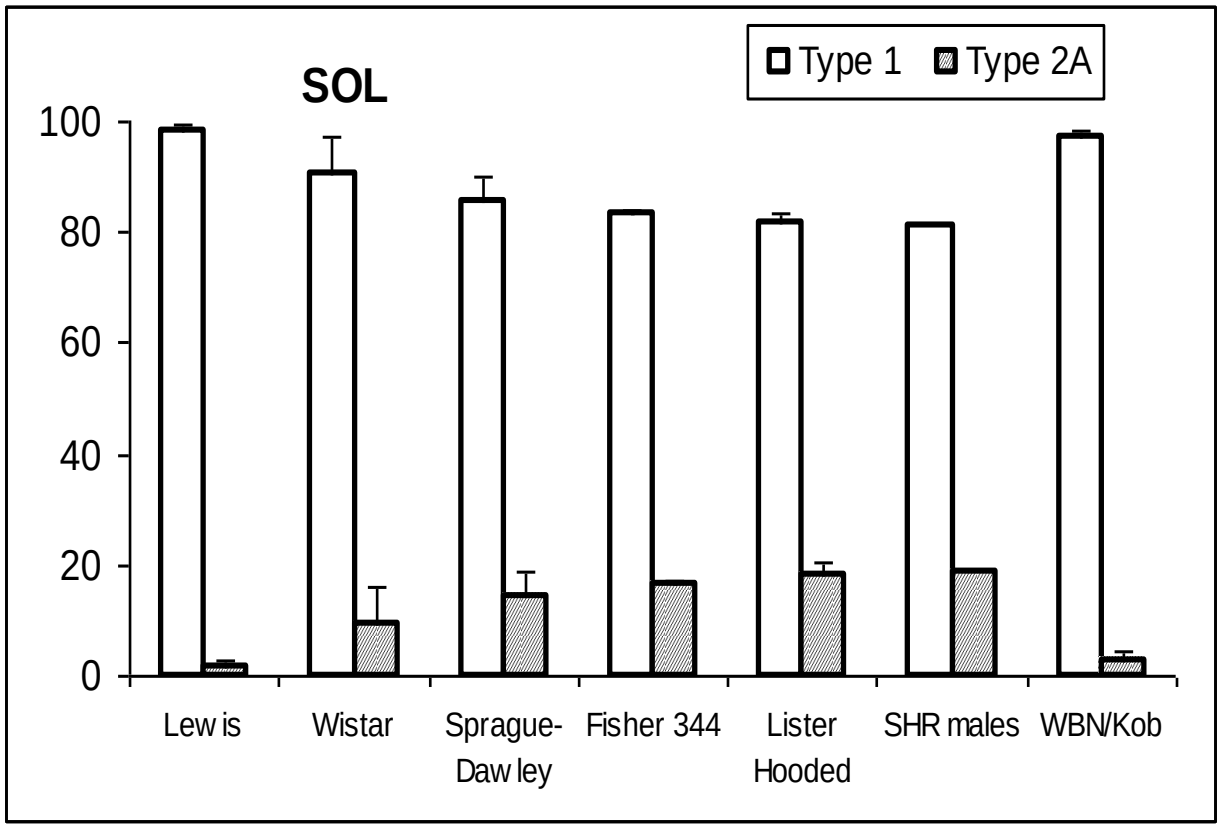
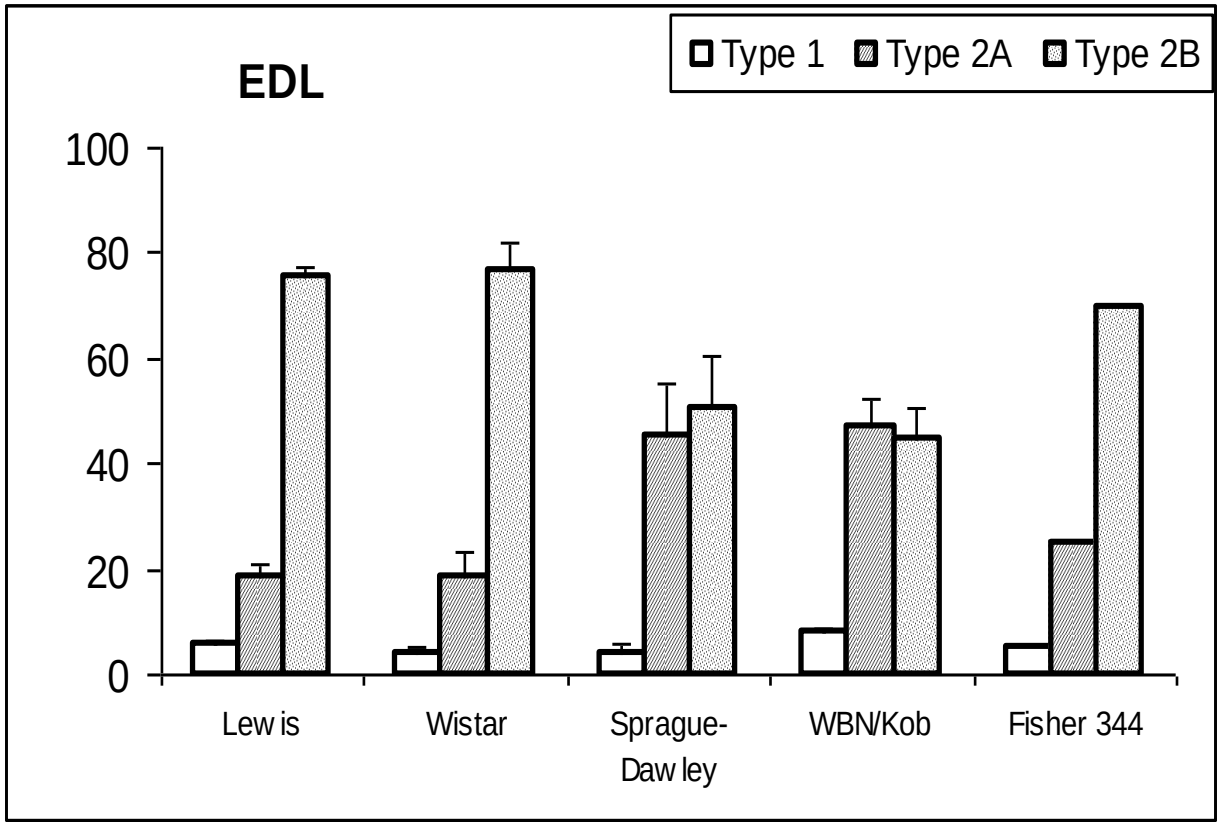

EDL
90
80
70
60
50
40
30
20
10
0
F3*
M3
F6-14*
M6-14
Type 1
Type 2A
Type 2B

Fig. 3
3A


SOL
Type 1
Type 2A
100
80
60
40
20
0
Lew is
Wistar
Sprague-Daw ley
Fisher 344
Lister Hooded
SHR males
WBN/Kob

3B


EDL
Type 1
Type 2A
Type 2B
100
80
60
40
20
0
Lew is
Wistar
Sprague-Daw ley
WBN/Kob
Fisher 344