# Fiber type composition of unoperated rat soleus and extensor digitorum longus muscles after unilateral isotransplantation of a foreign muscle in long-term experiments.

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**Summary**

We examined the effects of the unilateral heterochronous isotransplantation on the fiber type composition and myosin heavy chain (MyHC) isoform content of unoperated slow soleus and fast extensor digitorum longus muscles of female inbred Lewis strain rats. Comparison were made between "control" unoperated muscles of experimental, i.e. operated rats with the corresponding muscles of completely naive (unoperated) rats of 3 age groups (5-, 8- and 14-month-old). This was done in order to ascertain whether these muscles can be used as reliable controls to the transplanted and host muscles for our ongoing grafting experiments. The fiber type composition was determined by assessing the histochemical reaction for myofibrillar adenosine triphosphatase, the MyHC isoform content was determined immunocytochemically using monoclonal antibodies specific to different MyHC isoforms and by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Our experiments show that the heterochronous isotransplantation procedure had no significant effect on the fiber type composition and MyHC isoform content of the "control" unoperated muscles of the experimental rats when compared to the corresponding muscles of the naive animals. Further, the duration and type of isotransplantation also did not lead to differences among experimental muscles. We conclude that the unoperated muscles of the experimental animals can be used as controls in our current transplantation project dealing with long-term grafting experiments.

**Key words**: muscle transplantations – muscle fiber types – effect of surgical treatment – mATPase and immunohistochemistry - myosin heavy chains

**Introduction**

Within the European Muscle Network project (MYORES, Network of excellence no. 511978, Multi-organismic Approach to Study Normal and Aberrant Muscle Development, Function and Repair) we are studying the influence of innervation and thyroid hormones on the diversification of

muscle fiber phenotype and MyHC isoform gene expression in regenerated muscles. For this purpose, a method of heterochronous isotransplantation was successfully introduced in rats (Jirmanová and Soukup 1995). In this experimental model two types of muscle transplantation are performed; the soleus (SOL) or extensor digitorum longus (EDL) muscles of *young* inbred Lewis rats are intramuscularly grafted either into the host EDL or SOL muscles of *adult* animals of the same strain. If the isotransplantation does not affect the unoperated SOL and EDL muscles of experimental rats in the long-term experiments, these muscles could be used as controls in the evaluation of the expected fiber type transitions in transplanted and host muscles.

Though the experimental animals usually recovered soon after the transplantation and their mobility seemed to be unaffected, the possibility that the operated animals did not load both limbs to the same extent should not be ignored. The operated limb, with the host muscle additionally "loaded" with the muscle graft, might be less intensively used and loaded than the contralateral unoperated one, which might thus have been over-loaded. This could affect the MyHC isoform content and the fiber type composition of the control muscles, as has repeatedly been demonstrated that loading and un-loading induces fiber type transitions, especially in slow muscles (Asmussen and Soukup 1991,; Caiozzo *et al*. 1998, Goldspink *et al*. 1999,; for review see Hämäläinen and Pette 1995, Talmadge 2000,; Baldwin and Haddad 2001).

In our previous short-term study, performed on a smaller sample of young adult rats, we demonstrated that the unilateral isotransplantation into the EDL muscle had no significant effect on the fiber type composition and MyHC isoform content of the unoperated muscles even when compared to the homologous muscles of naive (unoperated) rats (Zachařová *et al*. 2005). However, possible long-term effects of these transplantations and altering experimental procedures on the "control" muscles were still possible. Therefore, in the present study, the fiber type composition and MyHC content of the unoperated muscles of the experimental animals were compared with that of the homonymous muscles of completely naive (unoperated) rats within the age range of 4 to 17 months. For the present

study, we used a much larger number of muscle samples, which enabled a statistical comparison of animals divided into three developmental age groups (the exact age is indicated in Methods and in Tables 1 and 2). An additional aim of this study was to ascertain whether altering (1) the type of muscle graft (SOL or EDL), (2) the location of the host muscle (right or left, or both), (3) the position of the control muscle according to the side of operation (ipsilateral or contralateral) or (4) the duration of the isotransplantation, has any effect on the control muscles in experimental rats.

Our data clearly show that the unilateral heterochronous isotransplantation procedure had no significant effect on the mean fiber type composition or mean MyHC isoform content of the "control" unoperated muscles of experimental rats when compared to the corresponding muscles of naive animals. The duration and the type of isotransplantation also did not lead to differences among the experimental muscles. Hence, the unoperated control muscles of the experimental animals can be used as controls in our current long-term grafting experiments in rats with altered thyroid status (Zachařová *et al*. 1999, Soukup *et al*. 2001, Vadászová *et al*. 2004, 2006a,b Vadászová-Soukup *et al*. 2006, Vadászová-Soukup and Soukup 2006, for review see Soukup and Jirmanová 2000).

**Methods**

*Muscle samples.* In this study SOL and EDL muscles of female inbred, 4- to 17-month-old rats of the Lewis strain were analyzed. The animals were obtained from the authorized laboratory of the rat-breeding unit of the Institute of Physiology (Accreditation number 1020/491/A/00). The maintenance and handling of the experimental animals followed the EU Council Directive (86/609EEC) and the investigation was approved by the Expert Committee of the Institute of Physiology of the Academy of Sciences, Prague, Czech Republic. Inbred rats are used to suppress the graft rejection and to facilitate the animals' survival. The muscles were collected after intraperitoneal injections of Nembutal (sodium pentobarbital, 40 mg/kg) and immediately frozen in liquid nitrogen, euthanasia was performed by an overdose of the anesthetic.

The SOL and EDL muscles were excised from both hind limbs of 20 naive and 21 experimental rats. In the experimental animals, heterochronous isotransplantation of SOL or EDL muscles, excised from 3- to 4-week-old rats, was performed into the right and/or left EDL or SOL muscle of adult 2- to 3-month-old rats (for details see Jirmanová and Soukup 1995, 2001; Soukup and Novotová 2000). Most of these transplants were unilateral, but a few were bilateral. Unoperated and experimental animals were divided into three age groups, for subsequent sampling, these corresponded to roughly 5-, 8- and 14 months (for exact age see Tables 1 and 2). Serial cryosections (10 μm) were prepared from each muscle belly and were analyzed for myofibrillar adenosine triphosphatase (mATPase) histochemistry and for MyHC immunocytochemistry. The remaining portions of the muscle were processed for SDS-PAGE.

*Myofibrillar ATPase-Histochemistry and Immunocytochemistry.* 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 (Guth and Samaha; 1970, Dubowitz and Brooke 1973). To reveal the MyHC isoform expression in muscle fibers, the muscle sections were incubated with different mouse monoclonal antibodies (mAbs) specific for rat MyHC isoforms BA-D5 (MyHC-1), SC-71 (MyHC-2a), F-35 (all MyHC except -2x/d) and BF-F3 (MyHC-2b) (Schiaffino *et al.* 1986). Additionally, mAbs anti Slow (MyHC-1) and anti Fast (MyHC-2), both provided by Biotrend, Medac/Novocastra or AbD Serotec were used to further distinguish slow and fast MyHC isoforms. The appropriate dilutions of mAbs (from undiluted to 1:1000 in PBS containing 5 % bovine or donkey serum albumin) were determined after testing of hybridoma culture supernatants by immunocytochemistry. Primary antibody binding was revealed by the standard indirect peroxidase-antiperoxidase (PAP) technique (Sternberger 1986) using the PAP products (Dakopatts, Copenhagen, Denmark), by the avidin-biotin method using Vectastain ABC (Vector Laboratories, CA, USA), or by using donkey secondary antibody conjugated with HRP (Jackson Immunoresearch Laboratories, USA).

*Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).* MyHC isoforms were either extracted as described earlier (Zachařová et al. 2005) or prepared as follows: muscle specimens were minced on ice and washed with five volumes of 20 mM NaCl, 5 mM sodium phosphate and 1 mM EGTA (pH 6.5). Myosin was extracted with 100 mM sodium phosphate, 5 mM EGTA and 1 mM dithiothreitol (pH 8.5) and the supernatant containing myosin was diluted with one volume of glycerol (87 %) and stored at – 20 º C (d'Albis et al. 1979). MyHC isoforms were separated by SDS-PAGE (Talmadge and Roy 1993). The separating gel contained 8 % acrylamide, 0.16 % bis-acrylamide, 37.4 % glycerol, 0.4 % SDS, 0.2 M Tris-base (pH 8.8), 0.1 M glycine, 0.1 % ammonium persulphate and 0.06 % tetramethylethylenediamine (TEMED). The stacking gel consisted of 4.2 % acrylamide, 0.08 % bis-acrylamide, 37.4 % glycerol, 0.4 % SDS, 70 mM Tris-base (pH 6.8), 4 mM EDTA, 0.1 % ammonium persulphate (APS) and 0.12 % TEMED. The inside running buffer consisted of 150 mM glycine, 100 mM Tris and 0.1 % SDS and outside buffer was twice diluted. Electrophoresis was carried out at constant voltage (70 V) for 30 h at 4 ºC. After MyHC isoform separation, the gels were silver-stained (Blum *et al*. 1987) and individual bands were densitometrically evaluated using Quantity One Program (of Bio-Rad Laboratories) or using the AIDA 3.28 computer program (Advanced Image Data Analyzer, Germany).

*Quantitative morphological analysis.* The numerical (N) and area (A) proportions (%) of muscle fiber types, determined according to the mATPase reaction and the MyHC isoform content were assessed by a 2-D stereological method using the principles of unbiased counting frame and point counting (Zachařová and Kubínová 1995, Zachařová *et al.* 1997, 2005). The stereological measurements were performed by the C.A.S.T. Grid System (Olympus, Albertslund, Denmark). The data are expressed as means ± S.D. and the significance ($p < 0.05$) was evaluated by a suitable statistical test (Mann-Whitney, Kolmogorov-Smirnov or T-test) depending on the distribution normality of experimental data analyzed by the Shapiro-Wilks test.

## Results

### *1. Fiber type composition and MyHC isoform content.*

As was demonstrated by mATPase reaction, the normal SOL muscles of the naive rats and the control SOL muscles of experimental rats were composed of two fiber types, type 1 and 2A; these were supplemented by scarce transitional or hybrid type 2C (1C) fibers (Fig. 1). Type 1 fibers were stained positively after acid preincubations at pH 4.3 and 4.5 for the mATPase reaction and with anti-slow and BA-D5 mAbs, while type 2A fibers were positively stained after the alkaline preincubation and with anti-fast and SC-71 mAbs. All fibers of both types were stained by BF-35 mAb, specific to all MyHC isoforms except -2x/d. Type 2C (1C) fibers were positively stained to a variable extent after acid and alkaline preincubations and, as expected, they co-expressed MyHC-1 and MyHC-2a isoforms. In examining the results of mATPase reaction and immunocytochemical reaction with BF-35 and BF-F3 mAbs, it is clear that type 2X/D and 2B fibers were not present in the SOL muscles of both naive and experimental rats (Fig. 1).

In the majority of the analyzed SOL muscles, SDS-PAGE revealed only MyHC-1 and -2a isoforms. However, contrary to the histochemical and immunocytochemical techniques, SDS-PAGE revealed traces of MyHC-2x/d or/and of -2b isoforms in four normal SOL muscles out of 37 muscles analyzed from the naive rats and in four SOL muscles out of 39 analyzed muscles from the experimental rats (Fig. 3a-e).

In the EDL muscles of naive and experimental rats, type 1, 2A and 2B fibers were demonstrated by mATPase reaction (Fig. 2 A-B, a-b), while four fiber types (type 1, 2A, 2X/D and 2B) were revealed by immunocytochemistry (Fig. 2 C-F, c-f). Type 1 and 2A fibers exhibited identical histochemical and immunocytochemical characteristics as in the SOL muscle and the histochemically defined 2B fibers showed a fast phenotype characterized by high mATPase activity after preincubation at pH 10.3 and moderate after preincubation at pH 4.5. Immunohistochemically, MyHC isoforms either -2x/d (the only negatively marked by BF-35 mAb) or -2b (positively stained by BF-F3 mAb) were demonstrated

in type 2X/D and 2B fibers, respectively. Similarly, four MyHC isoforms (MyHC-1, -2a, -2x/d and -2b) were demonstrated by the SDS-PAGE technique (cf. mixed sample in Fig. 3a).

***2. Quantitative comparison between muscles of naive and experimental rats.***

With a few exceptions, the statistical analysis of SOL and EDL muscles (by the paired T-test and by Mann-Whitney or Kolmogorov-Smirnov tests) revealed no significant differences in the mean fiber type composition and the mean MyHC isoform content between the right and left SOL and EDL muscles of naive and experimental rats. Therefore, for further comparative analysis of studied parameters between the naive and experimental rats and among the respective age groups, the data of left and right SOL and EDL muscles were pooled. *Firstly,* we found no effect of age, as there were no significant differences in the analyzed parameters among the three age groups either in the naive or the experimental rats (compare three age groups of either naive or experimental rats in Tables 1 and 2). *Secondly,* our data showed no significant differences in the analyzed parameters between corresponding muscles from experimental and naive animals (see Tables 1 and 2, Fig. 4). *Thirdly*, there were no significant differences in the analyzed parameters among any of the three age group of experimental and naive rats. *Fourthly,* the fact that the analyzed parameters of the three age groups of experimental rats were not statistically different shows that the duration of the post-operation period had no effect on the fiber type composition and MyHC content in the analyzed muscles (see the comparison in the three age groups of experimental rats in Tables 1 and 2)**.**

***3. The effect of different types of isotransplantation on the mean fiber type composition and the mean MyHC isoform content of control muscles in experimental animals.***

We found that the type of transplanted muscle (either SOL or EDL), as well as the type of the host muscle (either SOL or EDL) and the side of the transplantation (left or right) had no effect on the fiber type composition and MyHC isoform content of the control muscles. Furthermore, there were no

significant differences in the fiber type and MyHC content of control muscles between the contralateral and ipsilateral SOL or EDL muscles. Neither the duration of the isotransplantation (in average 3, 6 and 12 months) nor the unilateral or bilateral transplantation affected the analyzed parameters of unoperated control muscles.

**Discussion**

In the present study, we have demonstrated that i) there was no significant difference in fiber type composition and MyHC content of control (unoperated) SOL and EDL muscles excised from the experimental rats and of normal SOL and EDL muscles excised from the naive rats of the same age and ii) that there were no differences found when we compared any of the three age groups of experimental and naive rats. Furthermore, iii) the heterochronous isotransplantation surgery, regardless of the duration, the side of transplantation or the type of grafted and host muscle, had no significant influence on the fiber type composition and MyHC isoform content of the control (unoperated) contralateral or ipsilateral SOL and EDL muscles of the experimental animals at any age period examined. This clearly means that the composition of control muscles had not been affected by the duration and the type of isotransplantation (SOL/EDL, EDL/EDL, EDL/SOL or SOL/SOL), by the position in relation to the operated muscle (contralateral/ipsilateral), if the operated leg was on the left or right side or if the transplantation was unilateral or bilateral. We have found no statistically significant differences in our relatively large sample of muscles. There were, however, marked differences between left and right SOL muscles in some rats, as well as between individual rats. Nonetheless, our results demonstrate that the unoperated muscles of the experimental animals can be used, in general, as reliable controls for all types of regenerated muscle grafts and host muscles in our current analysis of thyroid hormone influence on the regulation of muscle phenotype (for review see Soukup and Jirmanová 2000).

*Comparison of different experimental factors.* In the long-lasting experimental study of muscle plasticity, various factors that may influence the fiber type composition and MyHC isoform content must be considered. In the present study, we have therefore compared a large sample of muscles from experimental and naive rats in order to avoid a possible effect of the interindividual variability, differences between the right and the left side and the age, as well as of the duration and the type of isotransplantation in the experimental animals.

*Firstly,* the fiber type composition of muscles may change during the life of animals. The comparison among the three age groups of adult naive and experimental rats showed that the SOL and EDL fiber type composition does not change significantly between the fourth and 17th month of age (cf. also Soukup *et al*. 2002). We have not included in this paper our results of fiber type composition and MyHC content of naive rats younger than 4 months, because (as already described) the isotransplantation was performed in about 2-month-old rats and the graft regeneration was allowed at least for another two months in order to achieve its full regeneration. This means that the youngest experimental rats, available for comparison were 4 months old. The maximal time allowed for muscle regeneration in our grafting experiments was 15 months, thus the oldest experimental animals were about 17 months old. Nevertheless, the comparison of the oldest animals with other age groups did not reveal any significant differences, although the 17-month-old rats can already be regarded as "old" ones.

*Secondly,* though the analyzed animals were of the inbred strain (Lewis), it is possible that an individual variability in the fiber type composition and MyHC content of homonymous muscles among different rats can play a role. A possible variability between the right and the left muscle can also be expected in some rats. Although there were no significant differences between the mean values of the right and the left side, in four naive and four experimental individual rats, the differences between the left and right SOL muscles were greater than 10 %. The differences between the EDL muscles were, in general, much smaller, even in rats with markedly different composition of the SOL

muscles. Furthermore, our results show that in the slow SOL muscle, in which MyHC-1 predominates, a certain degree of variability is possible even in the expression pattern of fast MyHC isoforms. As determined by the histochemical reaction for mATPase and by immunohistochemical reactions, the most commonly expressed fast isoform in SOL muscle is MyHC-2a. But using the SDS-PAGE technique we demonstrated that SOL muscles may also express even "uncommon" MyHC-2x/d and -2b isoforms. We assume that even uncommonly expressed fast MyHC isoforms may contribute to the interindividual variability of muscles, however, depending on proportion of their content in the muscle. It is important to note that if different rat strains are used for comparative studies, the existing strain differences also have to be considered (Soukup *et al*. 2002).

*Thirdly,* different experimental procedures may also affect the muscle fiber phenotype to a variable extent. However, our results do not suggest any influence of the duration of isotransplantation, as well as of minor differences among isotransplantation methods on the fiber type composition and MyHC isoform content in the "control" muscles of the experimental animals.

We therefore conclude that the control muscles of the experimental animals can be used as controls to the host and regenerated muscle grafts in our studies, where we are trying to evaluate the effect of various intrinsic (genetic) and extrinsic (innervation, thyroid hormone levels) factors on the differentiation of muscle phenotype.

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**References**

ASMUSSEN G, SOUKUP T: Arrest of developmental conversion of type II to type I fibers after suspension hypokinesia. *Histochem J* **23**: 312-322, 1991.

BALDWIN K, HADDAD F: Plasticity in skeletal, cardiac, and smooth muscle. Invited review: Effects of different activity and inactivity paradigms on myosin heavy chain gene expression in striated muscles. *J Appl Physiol* **90**: 345-357, 2001.

BLUM H, BEIER H, GROSS HJ: Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis* **8**: 93-99, 1987.

CAIOZZO VJ, BAKER MJ, BALDWIN KM: Novel transitions in MHC isoforms: separate and combined effects of thyroid hormone and mechanical unloading. *J Appl Physiol* **85**: 2237-2248, 1998.

D´ALBIS A, PANTALONI C, BECHET JJ: An electrophoretic study of native myosin isoenzymes and of their subunit content. *Eur J Biochem* **99**: 261-272, 1979.

DUBOWITZ V, BROOKE MH: Histological and histochemical stains and reactions. In: Dubowitz V, Brooke MH (eds), Muscle biopsy: A modern approach. Major problems in neurology, Vol. 2. WB Saunders, London, pp 20-33, 1973.

GOLDSPINK G: Changes in muscle mass and phenotype and the expression of autocrine and systemic growth factors by muscle in response to stretch and overload. *J Anat* **194**: 323-334, 1999.

GUTH L, SAMAHA FJ: Procedure for the histochemical demonstration of actomyosin ATPase. *Exp Neurol* **28**: 365-367, 1970.

HÄMÄLÄINEN N, PETTE D: Patterns of myosin isoforms in mammalian skeletal muscle fibres. *J Microsc Res Tech* **30:** 381-389, 1995.

JIRMANOVÁ I, SOUKUP T: Critical period in muscle spindle regeneration in grafts of developing rat muscle. *Anat Embryol* **192**: 283-291, 1995.

JIRMANOVÁ I, SOUKUP T: Early changes in extrafusal and intrafusal muscle fibres following heterochronous isotransplantation. *Acta Neuropathol* **102**: 473-484, 2001.

SCHIAFFINO S, SAGGIN L, VIEL A, AUSONI S, SARTORE S, GORZA L: Muscle fiber types identified by monoclonal antibodies to myosin heavy chains. In: Benzi G, Packer L, Siliprandi N (eds) Biochemical Aspects of Physical Exercise. Elsevier, Amsterdam, pp 27-34, 1986.

SOUKUP T, JIRMANOVÁ I: Regulation of myosin expression in developing and regenerating extrafusal and intrafusal muscle fibres with special emphasis on the role of thyroid hormones. *Physiol Res* **49**: 617-633, 2000.

SOUKUP T, NOVOTOVÁ M: Ultrastructure and innervation of regenerated intrafusal muscle fibres in heterochronous isografts of the fast rat muscle. *Acta Neuropathol* **100**: 435-444, 2000.

SOUKUP T, ZACHAŘOVÁ G, SMERDU V, JIRMANOVÁ I: Body, heart, thyroid gland and skeletal muscle weight changes in rats with altered thyroid status. *Physiol Res* **50**: 619-626, 2001.

SOUKUP T, ZACHAŘOVÁ G, SMERDU V: Fibre type composition of soleus and extensor digitorum longus muscles in normal female inbred Lewis rats. *Acta Histochem* **104**: 399-405, 2002.

STERNBERGER LA: *Immunocytochemistry.* 3rd ed., Wiley, New York, 1986.

TALMADGE RJ, ROY RR: Electrophoretic separation of the rat skeletal muscle myosin heavy-chain isoforms. *J Appl Physiol* **75**: 2337-2340, 1993.

TALMADGE RJ: Myosin heavy chain isoform expression following reduced neuromuscular activity: potential regulatory mechanisms. *Muscle Nerve* **23**: 661-679, 2000.

VADÁSZOVÁ A, ZACHAŘOVÁ G, MACHÁČOVÁ K, JIRMANOVÁ I, SOUKUP T: Influence of thyroid status on the differentiation of slow and fast rat muscle phenotypes. *Physiol Res* **53** (Suppl 1): S57-S61, 2004.

VADÁSZOVÁ A, HUDECOVÁ S, KRIŽANOVÁ O, SOUKUP T: Levels of myosin heavy chain mRNA transcripts and content of protein isoforms in the slow soleus muscle of 7-month-old rats with altered thyroid status. *Physiol Res* **55**: 221-225, 2006a.

VADÁSZOVÁ A, HUDECOVÁ S, KRIŽANOVÁ O, SOUKUP T: Levels of myosin heavy chain mRNA transcripts and protein isoforms in the fast extensor digitorum longus muscle of 7-month-old rats with chronic thyroid status alterations. *Physiol Res* **55**: 707-710, 2006b.

VADÁSZOVÁ-SOUKUP A, SMERDU V, ŽURMANOVÁ J, MALÁČOVÁ D, SOUKUP T: Unilateral foreign muscle transplantation has no effect on phenotype of unoperated rat muscles in long term experiments. *Physiol Res* **55**: 49P, 2006.

VADÁSZOVÁ-SOUKUP A, SOUKUP T: Dual role of thyroid hormones in the rat soleus muscle MyHC isoform expression. *Physiol Res* **56**: in press, 2007.

ZACHAŘOVÁ G, KUBÍNOVÁ L: Stereological methods based on point counting and unbiased counting frames for two-dimensional measurements in muscles: comparison with manual and image analysis methods. *J Muscle Res Cell Motil* **16**: 295-302, 1995.

ZACHAŘOVÁ G, KNOTKOVÁ-URBANCOVÁ J, HNÍK P, SOUKUP T: Nociceptive atrophy of the rat soleus muscle induced by bone fracture: a morphometric study. *J Appl Physiol* **82**: 552-557, 1997.

ZACHAŘOVÁ G, MRÁČKOVÁ K, JIRMANOVÁ I, SOUKUP T: Stereological evaluation of the soleus muscle isografted into fast extensor digitorum longus (EDL) muscle in rats with different thyroid status. *Gen Physiol Biophys* **18** (Suppl 1): 84-86, 1999.

ZACHAŘOVÁ G, VADÁSZOVÁ A, SMERDU V, ASMUSSEN G, SOUKUP T: The effect of a unilateral muscle transplantation on the muscle fiber type and the MyHC isoform content in unoperated hind limb slow and fast muscles of the inbred Lewis rats. *Physiol Res* **54**: 691-696, 2005.

**Table 1. Fiber type composition of soleus muscles**

Fiber type composition of the right and left soleus (SOL) muscles of naive (Norm) and experimental (Exp) 4 to 17-month-old rats, subdivided into 3 age groups. In the experimental rats, either the EDL or SOL muscle was isotransplanted into the host left or right EDL or SOL muscle. The data are expressed as percentages of slow type 1 fibers, demonstrated with mATPase reaction after acid preincubation at pH 4.3 or with anti slow (anti Slow) mAb and specific anti MyHC 1 (BA-D5) mAb and of fast type 2A fibers stained by mATPase reaction after alkaline preincubation at pH 10.3, with anti fast (anti Fast) mAb and specific anti MyHC-2a (SC-71) mAb. Note that there are no significant differences ($p < 0.05$) for any evaluated reaction between the SOL muscles of naive and experimental rats. n = number of muscles.

**Table 2. Fiber type composition of extensor digitorum longus muscles**

The mean content of fiber types of the right and left extensor digitorum longus (EDL) muscles of naive (Norm) and experimental (Exp) 4 to 17-month-old rats, subdivided into 3 age groups. In the experimental rats, either the EDL or SOL muscle was isotransplanted into the host left or right EDL or SOL muscle. The data are expressed as percentages of type 1, 2A and 2B fibers, classified according to the mATPase reaction or as percentages of fiber types determined by anti slow and anti fast MyHC mAbs and with specific mAbs recognizing MyHC-1 (BA-D5), -2a (SC-71), -2x/d (BF-35 as a negative marker) and -2b (BF-F3) MyHC isoforms. Note that no statistically significant difference ($p < 0.05$) was found for any evaluated reaction between the EDL muscles from naive and experimental rats. n = number of muscles.

**Fig. 1.** Fiber types and MyHC isoforms in the SOL muscles of naive 8-month-old (A-F) and of 9.5-month-old experimental (a-f) female inbred Lewis strain rats. To show fiber types the sections were stained for mATPase after acid preincubation at pH 4.3 (A1, a1), 4.5 (A2, a2) and after alkaline preincubation at pH 10.3 (B, b). MyHC isoforms were demonstrated with specific monoclonal

antibodies recognizing slow type 1 (BA-D5, C, c), 2a (SC71, D, d), 1+2a+2b (negative marker for 2x/d, BF-35, E, e) and 2b (BF-F3, F, f) MyHC isoforms. Note that there are no differences in reactions of naive and experimental animals. Bar = 100 μm.

**Fig. 2.** Fiber types and MyHC isoforms in the EDL muscles of the naive 8-month-old (A-F) and 9.5-month-old experimental (a-f) female inbred Lewis strain rats. The fiber types were determined according to the mATPase reaction after acid pH 4.3 preincubation (A1, a1), 4.5 (A2, a2) and 10.3 (C, c) and MyHC isoforms were revealed with monoclonal antibodies specifically recognizing slow type 1 (BA-D5, C, c), 2a (SC-71, D, d), 1+2a+2b (negative marker for 2x/d, BF-35, E, e) and 2b (BF-F3, F, f) MyHC isoforms. Note that there are no differences in reactions of naive and experimental animals. Bar = 100 μm.

**Fig. 3.** MyHC isoforms in the SOL muscles of normal 4-month-old (a-c) and experimental 12-month-old (d-e) female inbred Lewis strain rats demonstrated by SDS-PAGE and silver stained. In lane **a** all four MyHC isoforms in the order top-to-bottom: MyHC- 2a, -2x/d, -2b and -1 from a mixed sample of SOL and EDL muscle are shown as control to MyHC isoforms of SOL muscles (lanes b-e), containing different fast MyHC isoforms beside the predominating MyHC-1 isoform (the lowest band). In lane **b** SOL with commonly present MyHC-2a band is shown, in lanes **c-e** SOL muscles with "unusual" fast MyHC isoforms are presented: SOL with traces of MyHC-2x/d isoform (**c)**, SOL with MyHC-2b isoform (**d**) and SOL with MyHC-2x/d and -2b isoforms (**e**).

**Fig. 4A,B.** The mean content of type 1 and 2a MyHC isoforms in the rat SOL muscles (**A**) and of type 1, 2a, 2x/d and 2b isoforms in the EDL muscles (**B**) collected from hind limbs of naive (full columns) and experimental (after heterochronous isotransplantation, hatched columns) 4- to 17-month-old rats. Note that no statistically significant difference was found between naive and experimental rats. The number of analyzed muscles was 24 and 21 for SOL from naive and experimental rats, respectively and 32 and 28 for EDL from naive and experimental rats, respectively.

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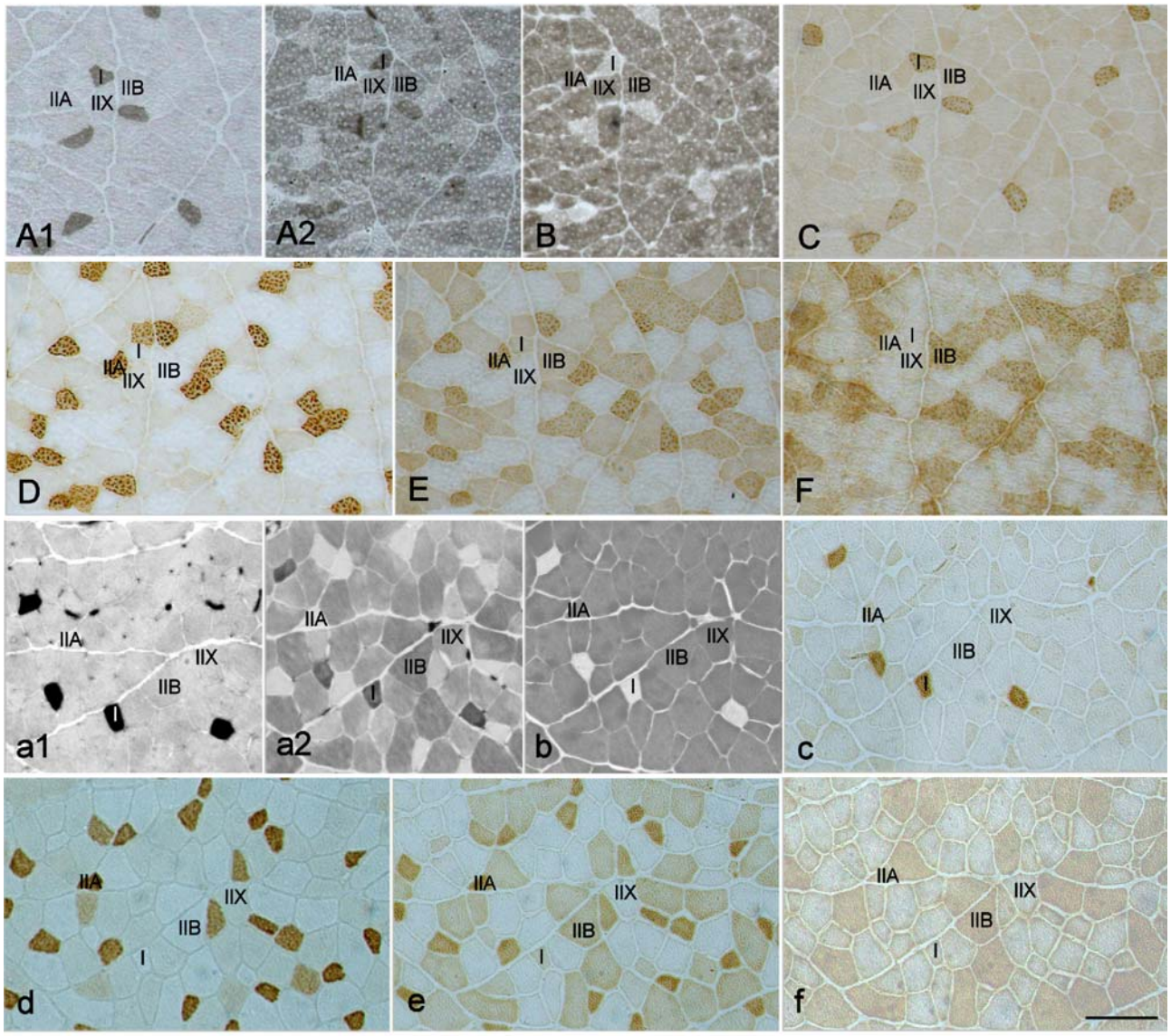

A1
I
IIA
IIX
IIB
A2
IIA
I
IIX
IIB
B
IIA
I
IIX
IIB
C
I
IIA
IIB
IIX
D
I
IIA
IIX
IIB
E
IIA
I
IIX
IIB
F
IIA
I
IIX
IIB
a1
IIA
IIX
IIB
I
a2
IIA
IIX
IIB
I
b
IIA
IIX
IIB
I
c
IIA
IIX
IIB
I
d
IIA
IIX
IIB
I
e
IIA
IIX
IIB
I
f
IIA
IIX
IIB
I

*Fig.2.*

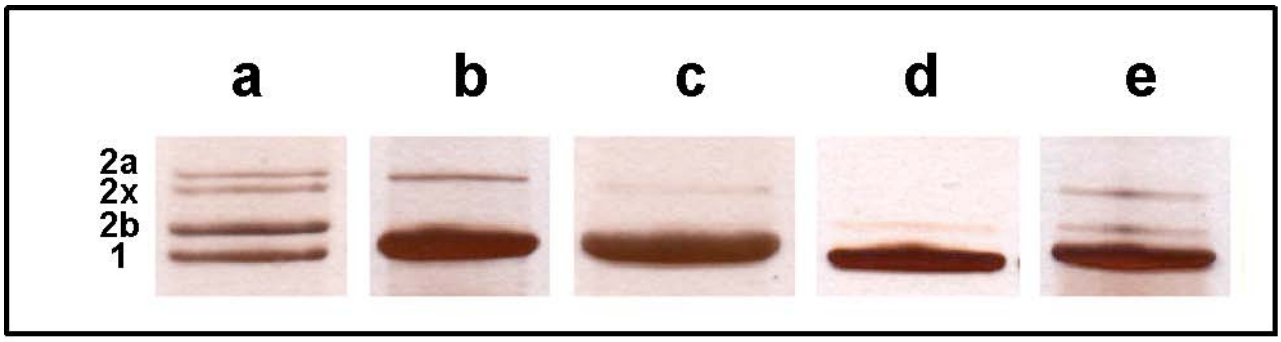
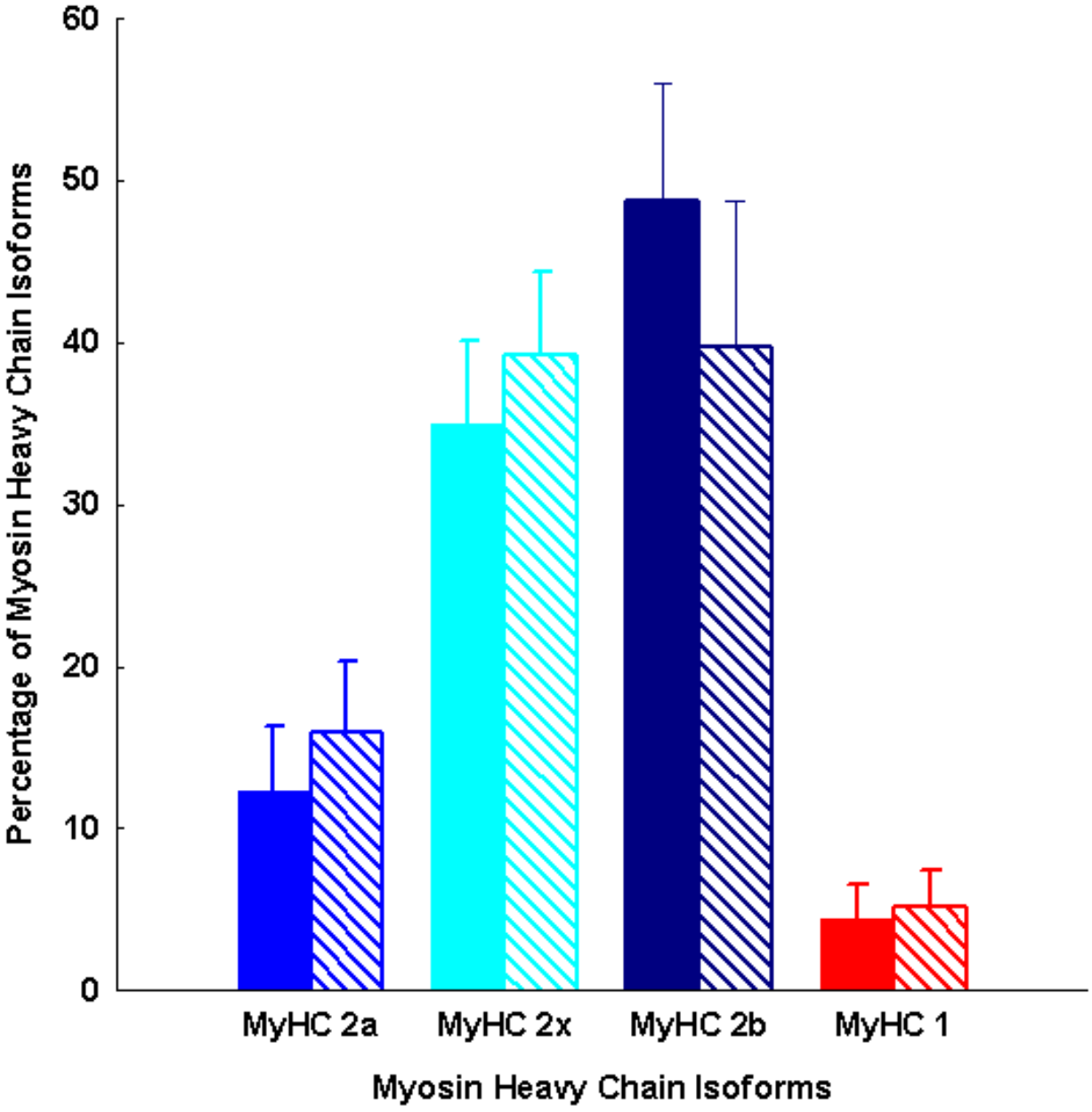



*Fig.3.*


60
50
40
30
20
10
0
Percentage of Myosin Heavy Chain Isoforms
MyHC 2a
MyHC 2x
MyHC 2b
MyHC 1
Myosin Heavy Chain Isoforms

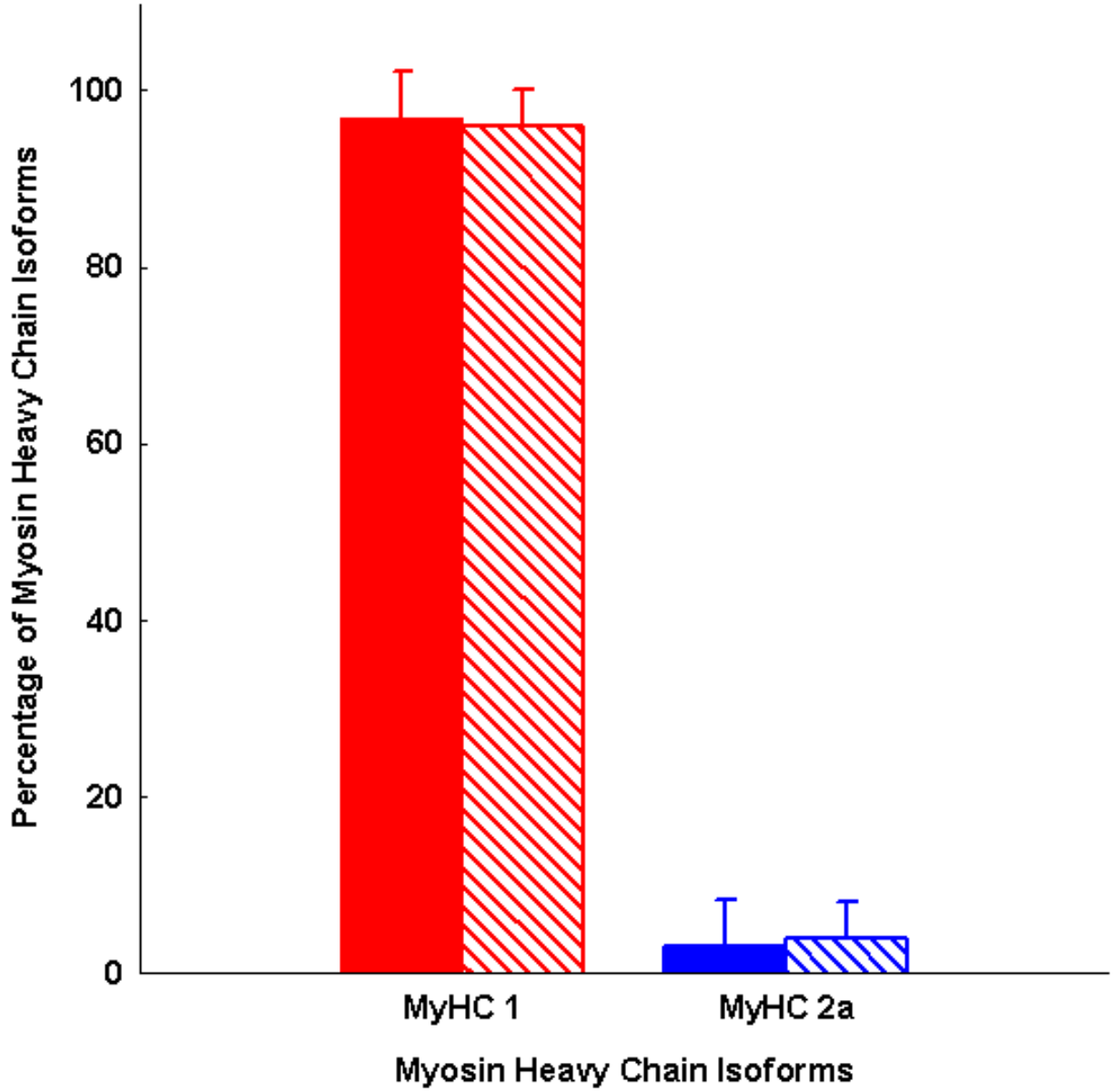



*Fig. 4A(bottom),4B (top)*

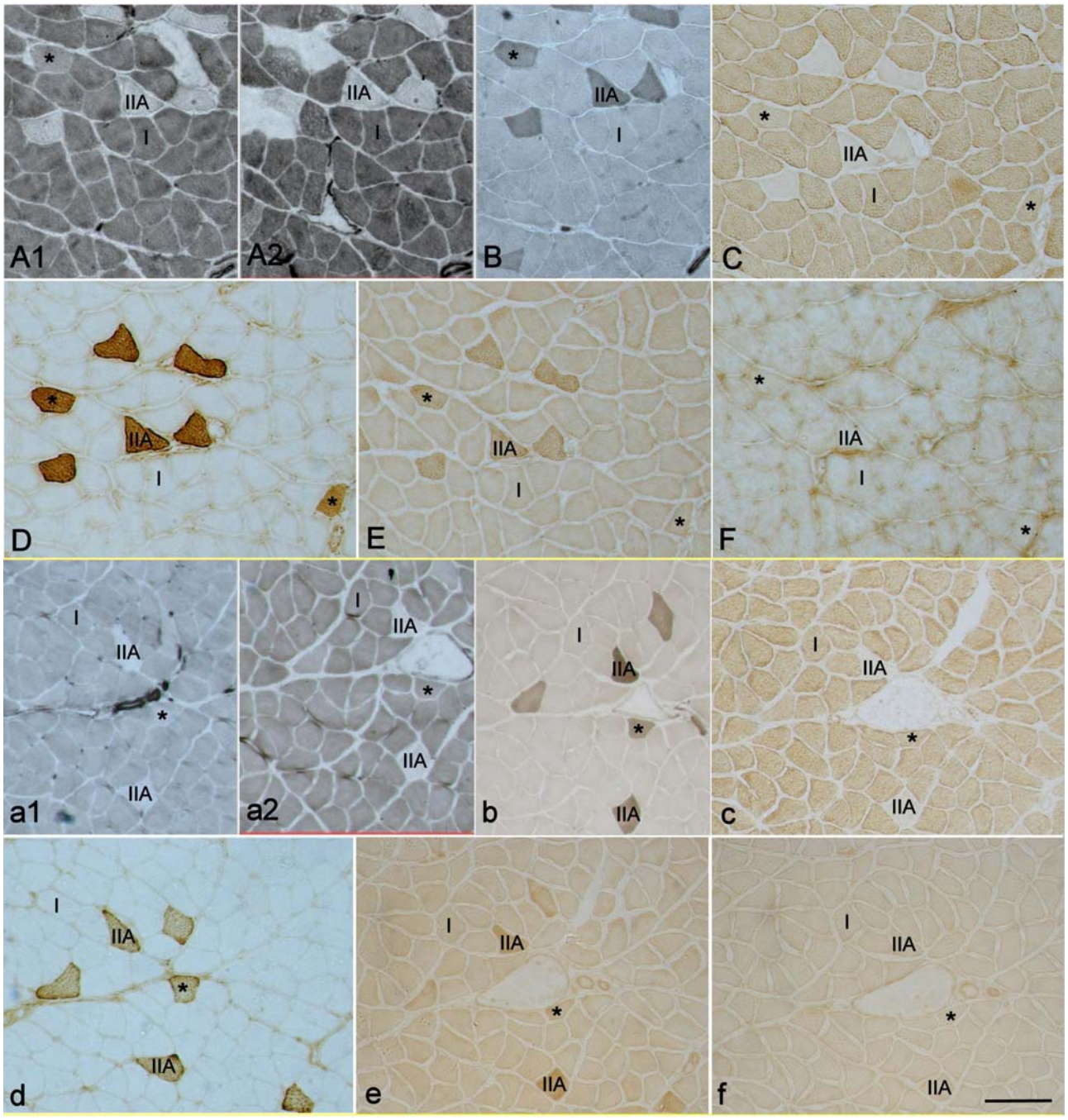



*Fig.1*

**Table 1. Fiber type composition of SOL muscles**

| | **Age group 1** *mean age ± S.D.* *4.8 ±0.9* | | **Age group 2** *mean age ± S.D.* *7.4 ±0.8* | | **Age group 3** *mean age ± S.D.* *14.1 ±2.3* | | **Total** (from all muscles) | |
|---|---|---|---|---|---|---|---|---|
| | **Norm** n=10 | **Exp** n=10 | **Norm** n=11 | **Exp** n=10 | **Norm** n=12 | **Exp** n=17 | **Norm** n=33 | **Exp** n=37 |
| **ATPase 4.3 type I fibers** | 98.4 *±2.6* | 98.5 *±2.2* | 97.3 *±3.0* | 98.5 *±2.4* | 97.8 *±2.7* | 99.1 *±1.5* | 97.8 *±2.7* | 98.6 *±1.9* |
| **ATPase 10.3 type 2A fibers** | 1.6 *±2.8* | 1.5 *±2.4* | 2.7 *±3.9* | 1.5 *±1.5* | 2.2 *±2.4* | 0.9 *±1.7* | 2.2 *±2.8* | 1.4 *±1.8* |
| **anti Slow MyHC I** | 97.8 *±2.6* | 99.0 *±2.1* | 96.8 *±2.8* | 98.3 *±1.5* | 97.7 *±2.9* | 99.0 *±2.2* | 97.4 *±2.1* | 98.8 *±2.2* |
| **anti Fast MyHC 2a** | 2.2 *±4.1* | 1.0 *±2.2* | 3.2 *±3.5* | 1.7 *±3.6* | 2.3 *±2.8* | 1.0 *±2.7* | 2.6 *±3.1* | 1.3 *±2.7* |
| **BA-D5 MyHC I** | 97.8 *±2.2* | 98.8 *±2.0* | 97.5 *±2.4* | 98.3 *±1.7* | 98.5 *±3.2* | 99.6 *±0.3* | 97.9 *±2.2* | 99.0 *±1.6* |
| **SC-71 MyHC 2a** | 2.2 *±3.2* | 1.2 *±2.5* | 2.5 *±3.0* | 1.7 *±2.5* | 1.5 *±3.2* | 0.4 *±0.7* | 2.1 *±3.0* | 1.0 *±2.0* |

**Table 2. Fiber type composition of EDL muscles**

| | **Age group 1** *mean age ± S.D.* *4.8 ±0.9* | | **Age group 2** *mean age ± S.D.* *7.3 ±0.8* | | **Age group 3** *mean age ± S.D.* *14.1 ±2.3* | | **Total** (from all muscles) | |
|---|---|---|---|---|---|---|---|---|
| | | *4.9 ±0.8* | | *8.9 ±0.7* | | *14.4 ±2.0* | | |
| | **Norm** n=10 | **Exp** n=10 | **Norm** n=12 | **Exp** n=10 | **Norm** n=10 | **Exp** n=9 | **Norm** n=32 | **Exp** n=29 |
| **ATPase 4.3 type I fibers** | 5.9 *±0.7* | 5.7 *±1.2* | 5.4 *±2.3* | 5.4 *±3.4* | 7.3 *±2.5* | 5.5 *±1.6* | 6.2 *±1.9* | 5.6 *±1.7* |
| **ATPase 4.5 type 2A fibers** | 16.9 *±3.7* | 17.2 *±2.3* | 18.3 *±3.8* | 17.0 *±4.2* | 16.2 *±2.5* | 19.6 *±3.0* | 17.2 *±3.4* | 18.2 *±3.1* |
| **ATPase 4.5 type 2B fibers** | 77.2 *±3.9* | 77.1 *±2.7* | 76.3 *±4.1* | 77.6 *±6.8* | 76.5 *±2.5* | 74.9 *±3.1* | 76.5 *±3.5* | 76.2 *±4.0* |
| **anti Slow type I MyHC** | 5.8 *±1.4* | 4.9 *±1.0* | 6.1 *±2.9* | 5.0 *±2.8* | 6.5 *±1.6* | 5.3 *±0.7* | 6.1 *±2.2* | 5.1 *±1.4* |
| **anti Fast 2a+2x/d+2b** | 94.2 *±1.4* | 95.1 *±1.4* | 93.9 *±1.5* | 95.0 *±4.2* | 93.5 *±1.6* | 94.7 *±0.9* | 93.9 *±2.9* | 94.9 *±1.6* |
| **BA-D5 MyHC I** | 5.4 *±0.6* | 5.8 *±2.6* | 6.1 *±3.2* | 4.8 *±2.8* | 7.3 *±2.5* | 4.8 *±0.8* | 6.2 *±2.1* | 5.2 *±2.0* |
| **SC-71 MyHC 2a** | 22.7 *±5.3* | 22.7 *±5.7* | 19.6 *±3.9* | 21.9 *±2.5* | 22.0 *±2.9* | 23.4 *±5.1* | 21.4 *±3.9* | 22.6 *±5.4* |
| **BF-35 MyHC 2x/d** | 29.4 *±6.1* | 28.4 *±2.9* | 34.3 *±4.7* | 33.1 *±6.8* | 30.0 *±6.4* | 34.2 *±8.3* | 31.4 *±5.9* | 32.2 *±6.9* |
| **BF-F3 MyHC 2b** | 42.5 *±6.5* | 43.1 *±4.4* | 40.0 *±8.8* | 40.2 *±6.4* | 40.8 *±3.4* | 37.6 *±8.4* | 41.0 *±6.1* | 40.2 *±7.3* |