PALACKÝ UNIVERSITY, OLOMOUC

MEDICAL FACULTY

DEPARTMENT OF PATHOLOGICAL PHYSIOLOGY

PhD THESIS

DETERMINATION AND REGULATION OF MYOSIN HEAVY CHAIN AND CALCIUM BINDING PROTEIN EXPRESSION IN SLOW AND FAST RAT MUSCLES

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CONTENT

I announce that I wrote the present work by myself and quoted all used literature and other related sources.

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1 LIST OF ABBREVIATIONS

CaBP-calcium binding protein

cCSQ-cardiac calsequestrin

CBB-Coomassie Brilliant Blue

DHPR-dihydropyridine receptor

EDL-extensor digitorum longus

EU-euthyroid

GAPDH- glyceraldehyde 3-phosphate dehydrogenase

GPDH-glycerol-3-phosphate dehydrogenase

GPDH:cyt c-glycerol-3-phosphate cytochrome c oxidoreductase

HY-hypothyroid

mATPase-myofibrillar adenosine triphosphatase

MHC-myosin heavy chain

NCX-Na^{+}/Ca^{2+} exchanger

PA-parvalbumin

PhL-phospholamban

RyR-ryanodine receptor

sCSQ-skeletal calsequestrin

SERCA-sarco/endoplasmic reticulum Ca^{2+}-ATPase

T_3-triiodothyronine

T_4-thyroxine

SR-sarcoplasmic reticulum

TH-hyperthyroid

TT-transverse tubule

2 INTRODUCTION

2.1 Fibre types and methods of fibre typing

Various classifications of fibre types have always been important for defining the physiological properties of skeletal muscles. MHC isoforms appear to be the best markers for muscle fibre type classification (Pette and Staron 2000a). According to their content, muscle fibres can be described as pure or hybrid. The pure fibres (1, 2A, 2X/D and 2B) contain just one MHC isoform, while the hybrid ones express more isoforms (Pette and Staron 2000a, Stephenson 2006). The MHC multigene family includes fast, cardiac and developmental isoforms. The fast isoforms (2A, 2X/D, 2B, superfast and extraocular) are present in 2A, 2X/D, 2B and masticatory or extraocular fast fibres, respectively. The cardiac isoforms (α and β) are predominantly expressed in cardiac muscles. The β isoform is also expressed in type 1 fibres, while the α isoform can be found e. g. in intrafusal fibres of muscle spindles (Pedrosa et al. 1990, for review see Soukup et al. 1995). The developmental isoforms (embryonic and perinatal/neonatal) can be found in regenerating and differentiating muscle fibres (Vadászová et al. 2004).

Changes in MHC expression caused e. g. by various factors like mechanical uploading and unloading, altered thyroid states, changed neuromuscular activity and aging lead to muscle fibre type transitions (Pette and Staron 2000a). This process is accompanied by an increased proportion of hybrid fibres. The transitions of MHC expression usually occur according to this pattern: 1↔ 1/2A ↔ 2A ↔2A/2X/D ↔ 2X/D ↔ 2X/D/2B ↔ 2B (Stephenson 2006).

In the past, different classifications of fibre types were used. In 1873, Ranvier described red and white muscle fibres. After the paper by Bárány (1967) demonstrating that actomyosin ATPase activity can be correlated with the speed of contraction, the histochemical demonstration of mATPase activity (Padykula and Herman 1955) became the most popular method of fibre 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 1 and fast type 2 fibres, the acid preincubation at pH 4.5 enables the further division of fast fibres into type 2A and 2B fibres. Thus the fibres that are stained positively after acid preincubations at pH 4.3 and

4.5 of the mATPase reaction are classified as type 1 fibres, while the fibres 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 fibres and the fibres characterised by high mATPase activity after preincubation at pH 10.3 and by moderate staining after preincubation at pH 4.5 are 2B fibres. Beside these "pure" fibre types, type 1C and 2C fibres, 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, 2000b, 2001, Stephenson 2006). Although the classification using mATPase reaction was overcome by the aforementioned modern division into four 1, 2A, 2X/D and 2B immunohistochemical fibre 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 2000b, 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 fibre type composition of mammalian skeletal muscles.

The slow soleus muscle and the fast EDL muscle apparently belong to the most frequently analysed 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 (Fig. 1) is composed of a great majority of slow type 1 fibres and of a variable, but usually low number of 2A fibres. On the other hand, the fast EDL muscle (Fig. 2) is, according to mATPase, composed of three histochemical muscle fibre types, i.e. of a low number of slow type 1 and of variable proportions of fast 2A and 2B fibres (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 fibre type analysis can be affected by

differences among individual rats and by the age or sex of the analysed animals. Recently, fibre type composition of the soleus and EDL muscles in 4- to 17-month-old female inbred Lewis strain rats was described (Soukup et al. 2009), but reliable analysis of male rats was lacking.

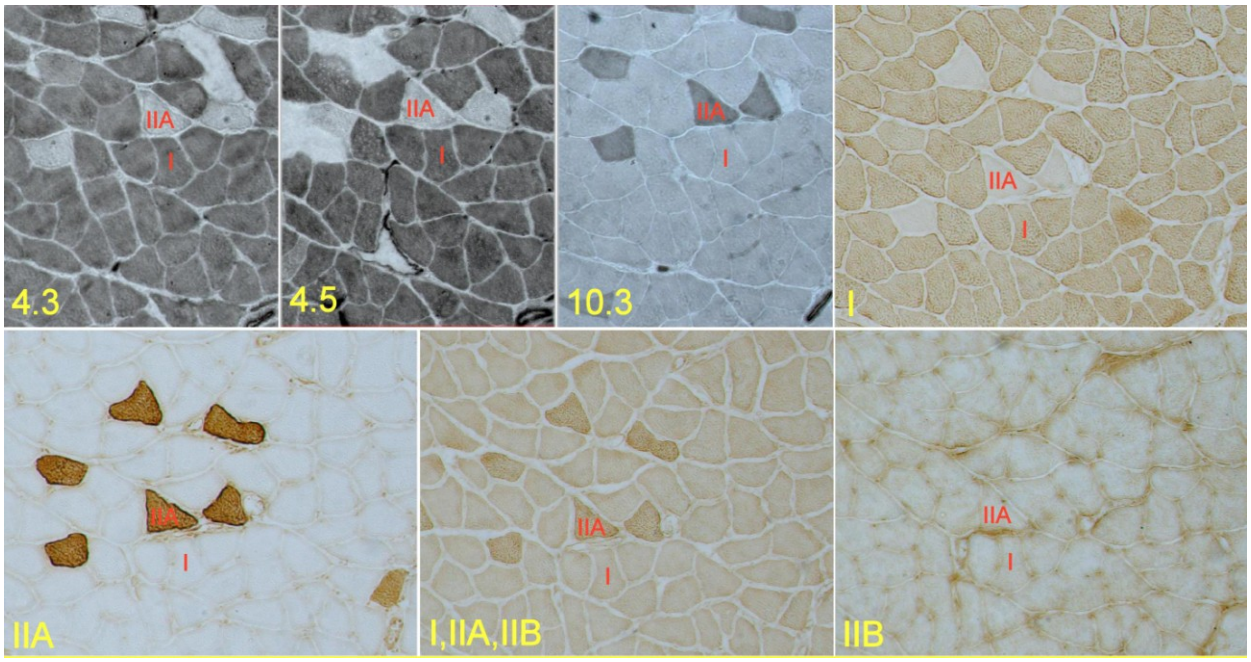

Fig. 1. An example of rat soleus muscle stained for mATPase at different pH (4.3, 4.5 and 10.3) and with monoclonal antibodies against 1, 2A, 2X/D and 2B MHC isoforms. Note that the soleus muscle contains only 1 and 2A isoforms as no 2X/D are revealed with BF-35 antibodies (these fibres should be negative) and with BF-F3 antibodies, no 2B fibres are stained.

Note: The figure was published by Soukup et al. (2009).

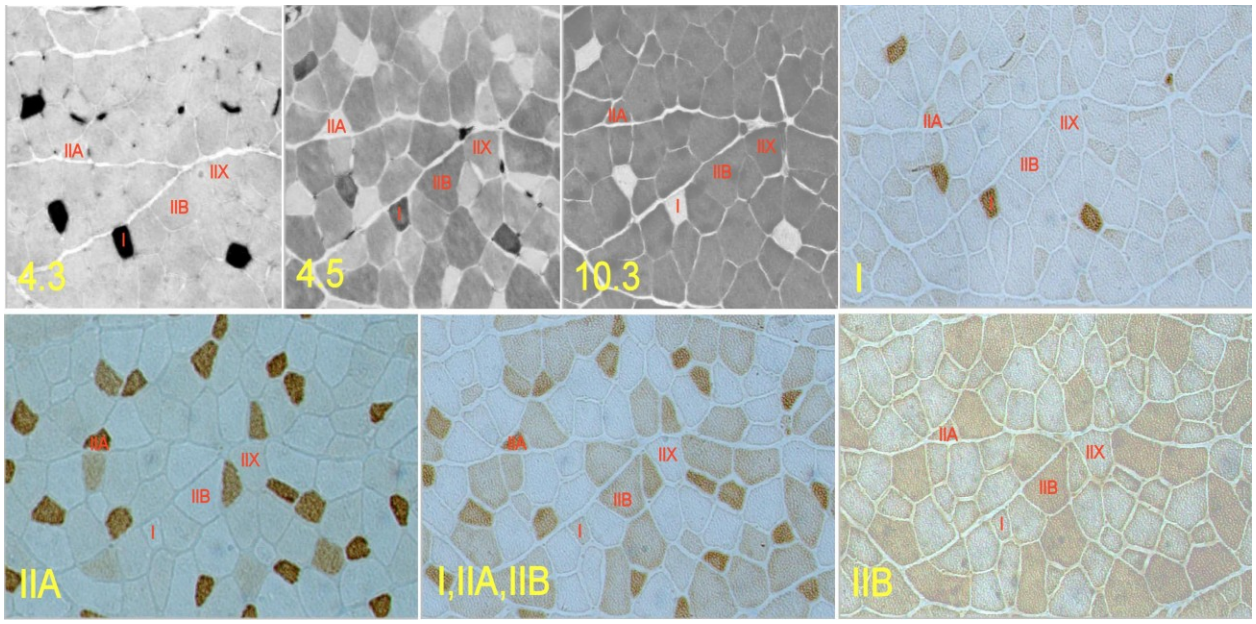

Fig. 2. Rat EDL muscle stained for mATPase at different pH (4.3, 4.5 and 10.3) and various MHC isoforms: 1, 2A, 2X/D (negative marker) and 2B.

Note: The figure was published by Soukup et al. (2009).

2.2 CaBPs and thyroid hormones in muscles

Ca^{2+} is one of the most important signalling molecules involved in various cellular processes (for review see Carafoli 2002, Berridge *et al.* 2003, Clapham 2007). It can be bound by many CaBPs (for review see Berchtold et al. 2000) that function as Ca^{2+} effectors, sensors or buffers and about 200 CaBPs are encoded by the human genome (Carafoli et al. 2001).

Several studies of mRNA and protein changes in the TH and HY states suggest that CaBPs of the SR responsible for calcium release and uptake (DHPR, RyR, SERCA, NCX) are all changed accordingly and coordinately regulated in response to the thyroid hormone level in both heart and skeletal muscles. The aforementioned CaBPs were increased in the TH and decreased in the HY status after acute 4- or 8-day (Arai et al. 1991) or chronic exposition (Hudecová et al. 2004).

CSQ is the most abundant CaBP in the SR of skeletal and cardiac muscle (MacLennan et al. 1983, for review see Beard et al. 2004, Novák and Soukup, submitted). It is an acidic protein that binds Ca^{2+} with moderate affinity and high capacity. It is

located in the SR lumen in close proximity to the junctional SR domains containing RyRs (Jorgensen et al. 1983, Franzini-Armstrong et al. 1987) thus keeping the free Ca^{2+} concentrations relatively low, which is important for easier and more efficient inward transport of the released calcium by SERCA pumps. CSQ is a part of the macromolecular complex (Fig. 3) involved in excitation-contraction coupling, the process linking surface membrane depolarisation to Ca^{2+} release from the SR (for review see Sandow 1965, Dulhunty 2006). CSQ is produced in a skeletal and a cardiac isoform, which are products of two different genes. The skeletal isoform (sCSQ, CSQ1) is found in fast-twitch and slow-twitch muscles, while the cardiac isoform (cCSQ, CSQ2) is the only transcript in cardiac muscle and a minor transcript in adult slow-twitch muscle (Fliegel et al. 1987, Scott et al. 1988, for review see Berchtold et al. 2000). Because cardiac and skeletal muscles differ in the content of CSQ isoforms, it can be presumed that the expression of cCSQ and sCSQ due to thyroid hormone alterations could be regulated in a different manner.

Studies related to CSQ changes in animals with the altered thyroid status are rare. It was only reported that the acute changes of the thyroid status for 4 and 8 days in the rabbit soleus might suggest that hyperthyroidism increased expression of sCSQ compared to cCSQ, while hypothyroidism had rather the opposite effect (Arai et al. 1991).CSQ changes after prolonged alteration of the thyroid status are completely unknown.

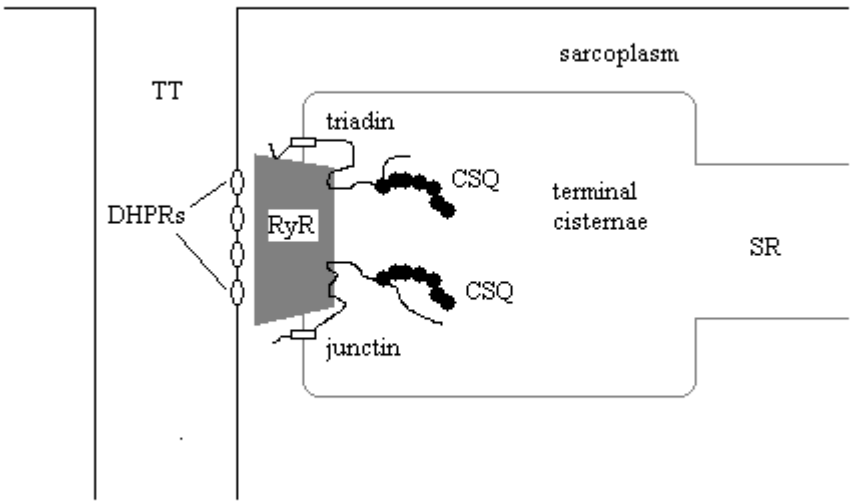

Fig. 3. CSQ in the lumen of the terminal cisternae of the SR forming a complex with triadin, junctin and RyR in a skeletal muscle fibre (Modified according Beard et al. 2009).

Opposite to CSQ, PA is a high-affinity CaBP found at high concentration only in fast-contracting/relaxing skeletal muscle fibers of vertebrates. In rat and mouse, the fastest type 2B fibres show the strongest immunoreactivity for PA. The majority of type 2A fibres (60-70%) exhibit moderate staining intensity and the remaining type 2A and the type 1 fibres lack PA. The majority of experimental data shows that this 12 kDa protein functions as a relaxing factor in the specialised fast-twitch muscle (Berchtold et al. 2000). PA is not essential as knockout mice grow and breed normally. They are also indistinguishable from wild-type mice with respect to their behaviour and physical activity under standard housing conditions (Schwaller et al. 1999). There is a close correlation between the PA content and relaxation speed of a variety of muscles. The concentration of PA is higher in smaller animals, which have higher contraction-relaxation speeds (Heizmann et al. 1982).The expression of of PA is greatly enhanced by phasic and drastically decreased by tonic motor-neuron activity (Leberer et al. 1986).

PA is supposed not to be affected by thyroid hormones in short term experiments, as no PA changes were revealed after 4-week treatment in TH and HY states (Müntener et al. 1987). Long term effects of thyroid state alterations, however, remain an open question.

PhL is a CaBP present in cardiac and slow skeletal muscles. This single-pass membrane protein inhibits SERCA, an ATP-driven pump that translocates calcium ions into the lumen of the SR, initiating muscle relaxation (Berchtold et al. 2000, Traaseth et al. 2008).

It was shown that there is quite a remarkable correlation between thyroid hormone action and GPDH activity and GPDH activity thus has become a very sensitive marker of the thyroid hormones status (Ruegamer et al. 1964, Okamura et al. 1981, Dümmler et al. 1996). In order to evaluate the effect of long term administration of thyroid hormones we have analysed the correlation between the GPDH activity or level and chronic (several-month) alteration of the thyroid hormone status.

It is worth to note the difference between heart and skeletal muscles exposed to increased levels of thyroid hormones. While in hearts hyperthyroidism leads to cardiac hypertrophy due to the increased haemodynamic load, skeletal muscles are not subjected to this change imposed on the heart. On the other hand, skeletal muscles react to thyroid hormone alteration by modification of their fibre type composition and MyHC content (d`Albis and Butler-Browne 1993, Larsson et al. 1994, Vadászová et al. 2006a, b, Vadászová-Soukup and Soukup 2007, Soukup et al. 2009, for review see Soukup and Jirmanová 2000). As mentioned above, changes in MHC expression caused by various factors (including altered thyroid states) lead to muscle fibre type transitions (Pette and Staron 2000a, Soukup and Jirmanová 2000). We can expect that these transitions are accompanied by changes of some other proteins which is necessary for changes of the muscle fibre function, These proteins should include various CaBPs as they regulate Ca^{2+} release and recovery and the speed of contraction and muscle relaxation.

3 GOALS OF THE STUDY

The first goal of the presented work was to analyse the contribution of individual, age, sex and strain differences to the variability of muscle fibre type composition. It included:

i) description of the fibre type composition of 3- 6-, 9- and 14-month-old age groups (range 3 to 19 months) of inbred Lewis rats of either sex,

ii) comparison of individual differences among animals in each experimental group,

iii) comparison of Lewis female and male rats of the same age ,

iv) comparison of the data on inbred Lewis rats, both male and female, with available literature data on other rat strains of corresponding age and sex.

The second goal of this work was to investigate chronic effects of thyroid hormone alterations on expression of CSQ, PA and PhL in fast and slow muscles of EU, HY and TH adult Lewis strain rats.

The third goal was to demonstrate reliable methods for evaluating the altered thyroid status. It included the examination of the following:

i) changes in the activity of GPDH in EU, HY and TH rats,

ii) changes of anatomical parameters (body, heart and thyroid gland weights) in EU, HY and TH rats.

4 MATERIAL AND METHODS

4.1 Animals and sample preparation

All experiments were performed on inbred Lewis strain rats obtained from the authorised 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). housed at 23±1 °C and at 12-hour light-dark cycle periods (6:00 a.m. to 6:00 p.m.) with *ad libitum* access to water and a complete laboratory diet. 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 EDL muscles used for analysis of fibre type composition 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..We used 5 to 9 rats in each group for the analysis of CSQ and 11, 12 and 11 rats for the analysis of, PA, PhL in soleus muscles and PhL in left ventricles, respectively. The animal groups used for GPDH activity analysis included female and male EU (n=29, mean age 8.7±3.9 months), HY (n=19, mean age 9.3±3.5 months) and TH rats (n=24, mean age 7.8±3.1 months). In all three experiments, the animals were anaesthetised with intraperitoneal injections of 1ml (100 mg) of Narketan (Ketaminum ut hydrochloridum) per 1000 g of body weight, followed by 0.5 ml (10 mg) of the myorelaxant Xylapan (Xylazinum ut hydrochloridum) per 1000 g of body weight. They were sacrificed by an overdose of the anaesthetic.

4.2 mATPase reaction

Muscle fibre types were determined according to the activity of mATPase 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 fibre 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).

4.3 Quantitative morphological analysis

The numerical proportions (%) of muscle fibre 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) was chosen according to the muscle section size and fibre 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 significance of differences between groups was evaluated by the t-test and/or Mann-Whitney test.

4.4 Alteration of the thyroid status

The TH was induced at 4 weeks and maintained for 3- to 9 months by intraperitoneal injections of 3,3',5-triiodo-L-thyronine (sodium salt, 0.15 mg/kg body weight) three times a week. The HY status was induced at 4 weeks and maintained for 4 to 11 months with a 0.05 % solution of methimazole (2-mercapto-1-methylimidazole) in drinking water. The EU rats were age-matched littermates of the experimental animals.

4.5 Measurement of GPDH activity and manipulation of livers, hearts, thyroid glands and blood samples

Excised livers from each animal were immediately processed for isolation of mitochondria. The enzyme activity was measured spectrophotometrically as GPDH:cyt *c* at room temperature following the reduction of cytochrome *c* at 550 nm.

Hearts and thyroid glands were quickly weighed; serum was prepared from blood samples by centrifugation for 15 min (1260 x g at 4 ºC) and frozen before it was used for measurements of total T_3 (tT_3) and total T_4 (tT_4) levels by a radioimmunoassay using commercial RIA kits (Immunotech - Beckman Coulter Co., Prague, Czech Republic) in collaboration with RNDr. Stanislav Pavelka, CSc. All measurements of GPDH activity were performed in the laboratory of RNDr. Hana Rauchová, CSc. and are described in a collaborative paper. For detailed description of the methods therefore see Rauchová et al. (2010).

4.6 CaBP SDS-PAGE and immunoblotting

Muscles were mechanically homogenised in cold buffer and then centrifuged. The collected supernatant was boiled with SDS-sample buffer (Laemmli 1970) for 5 min. One-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE), electrophoretic transfer of separated protein onto nitrocellulose membranes and details of the immunostaining procedure are described elsewhere (Dráber et al. 1988). CaBPs were revealed by specific monoclonal antibodies produced by Sigma (CSQ: C0743, PA: P3088) and Pierce (PhL: MA3-922). Bound antibodies were detected after incubation of the blots with secondary HRP-conjugated antibodies and after incubation with chemiluminescence reagents (Pierce). Exposed autoradiography films X-Omat AR (Eastman Kodak) with CSQ images, PA and PhLimages captured with LAS-1000 (Fuji) and photos of gels stained with CBB were quantified by densitometry using AIDA 3.21 Image Analyzer software (Raytest, Germany). The results are expressed in arbitrary units adjusted to CBB staining and GAPDH expression. The CSQ analysis was performed in collaboration with Vadym Sulimenko, PhD.

4.7 Western blot analysis of GPDH

Western blot analysis of GPDH was done in collaboration with RNDr. T. Mráček and is described in detail in the paper by (Rauchová et al. 2010). Briefly, tricine-SDS-PAGE electrophoreses of the samples were performed on 12% polyacrylamide slab gels (Mini Protean III, Bio-Rad, USA). Proteins from the gels were blotted onto PVDF membranes (Millipore) by semi-dry electrotransfer at 0.7 mA/cm2 for 1 h. The membranes were blocked in 10% (w/v) non-fat dry milk in TBS (150 mM NaCl, 10 mM Tris, pH 7.5). After blocking, they were incubated for 2 hrs in TBS, 0.1% (v/v) Tween 20 containing one of the following primary antibodies: rabbit polyclonal anti-GPDH antibody (Mráček et al. 2005, 1:5000 dilution) raised against the C-terminal peptide LDRRVPIPVDRSCGG of mouse enzyme according to Ueda et al. (1998) or mouse monoclonal cytochrome c oxidase subunit 4 (COX IV) (1:1000, MS407, Mitosciences, USA). Then, the membranes were incubated for 1 h using fluorescent secondary antibodies (goat antimouse IgG, 1:3000, Alexa Fluor 680 A-21058 or goat anti-rabbit IgG, 1:3000, Alexa Fluor 680 A-21109, Invitrogen, USA). The fluorescence was detected on an ODYSSEY system (LI-COR) and the signal was quantified using AIDA 3.21 Image Analyzer software (Raytest, Germany).

5 RESULTS

5.1 Fibre type composition

5.1.1 Fibre type composition and individual variability.

By the stereological method we analysed all muscle fibres in the cross sections through the muscle mid-belly (up to 2700 in the soleus and up to 4000 fibres 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 fibres were classified as type 1 (including 1C) fibres, the rest were 2A (including 2C) (Figs. 4, 6, Supplement 1). The analysed soleus muscles i) were composed exclusively of pure type 1 fibres exhibiting high acid-stable and low alkali-stable mATPase activity (12.2 %), ii) contained practically100 % of type 1 fibres and from these, just few (1-10) fibres exhibited high dual mATPase activity thus corresponding to 1C fibres (35.1 %), iii), contained a great majority of type 1 fibres (95-99.9 %) supplemented by a small number of 2A (2C) fibres (36.6 %) or iv) contained a majority of type 1 fibres, but more than 5.5 % (up to 12.7 %) of type 2A (2C) fibres (16.1 %). The content of type 1 fibres thus varied between 87.3 to 100 % and that of 2A fibres varied from zero to 12.7 %, which demonstrates a considerable individual variability of the soleus muscle in the inbred Lewis strain rats.

We have analysed 129 EDL muscles, 94 from female and 35 from male rats. All the EDL muscles contained type 1, 2A and 2B fibres as determined on the basis of mATPase activity after acid preincubation at pH 4.5. The average number of 2B fibres in all the examined EDL muscles greatly outnumbered 2A fibres and the number of type 1 fibres was invariably the lowest (Figs. 5, 7, Supplement 2). This fibre type composition was characteristic for all EDL muscles, although a certain degree of variability occurred as well. Each fibre type contributed to the individual variability to the similar extent, but proportions of 2A and 2B fibres varied most frequently.

We have not specifically searched for hybrid fibres (1C and 2C with the positive staining after both acid and alkali preincubations), as the stereological method does not compare individual fibres in more reactions. Analyses of fibres with acid-stable (type 1) and alkali-stable (2A, 2B) mATPase activity on serial sections showed that the average

percentage of hybrid fibres 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 %).

Note: The presented results were published (Novák et al. 2010b).

5.1.2 Age differences in fibre type composition

We have analysed the fibre 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 the older groups revealed a significant difference in the fibre type composition of the soleus (Fig. 4), but not of the EDL muscles (Fig. 5). In the youngest group we found that about 70 % of soleus muscles contained a variable percentage of 2A fibres, but no muscle was composed purely of type 1 fibres. On the other hand, in older age groups almost 80 % of analysed soleus muscles in females and more than 90 % in males were solely composed of type 1 (1C) fibres. When we compared the 6-, 9- and 14-month-old rats, we found no significant difference in fibre 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 fibres compared to the F6 and F14 groups in the EDL muscle (these differences are at the border of significance) (Fig. 5).

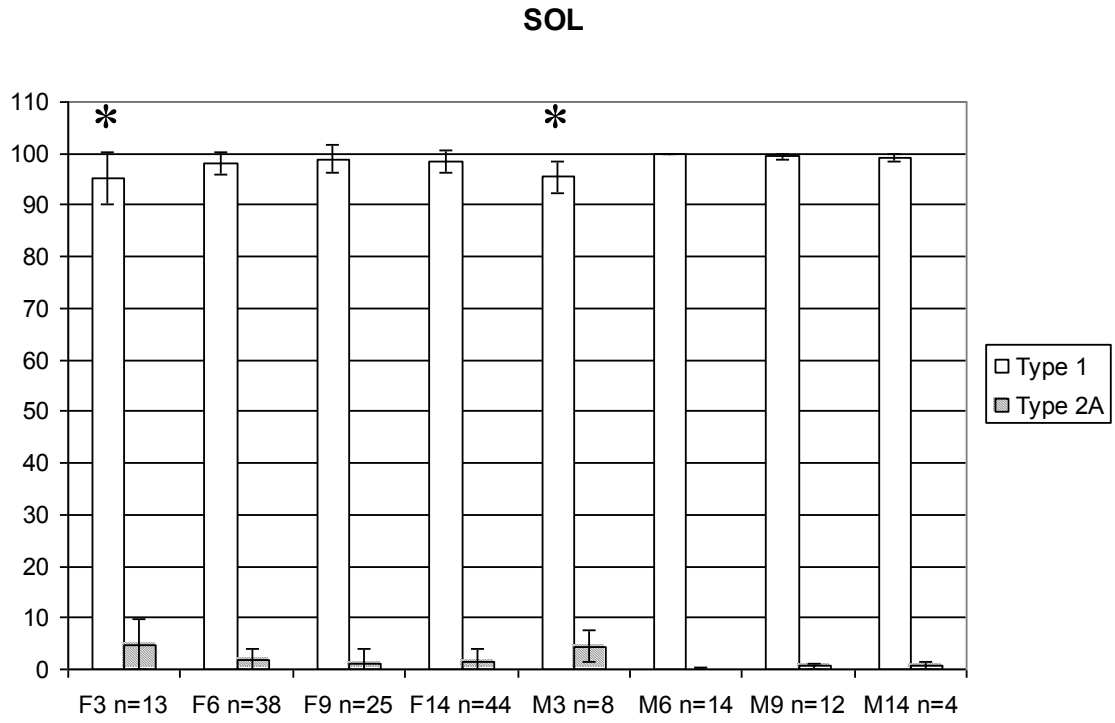

Fig. 4 Age differences in the fibre type composition of the soleus (SOL) muscle in female (F) and male (M) postnatal inbred Lewis strain rats in four age groups. Numerals on the x axis indicate age in months, n indicates the number of muscles analysed. Note that both female and male 3-month-old rats exhibit a significantly lower percentage of type 1 fibres when compared with the older animals (* indicates a significant difference, $p < 0.05$). Note also that there is no significant difference in fibre type composition among 6-, 9- and 14-month-old age groups of both female and male rats.

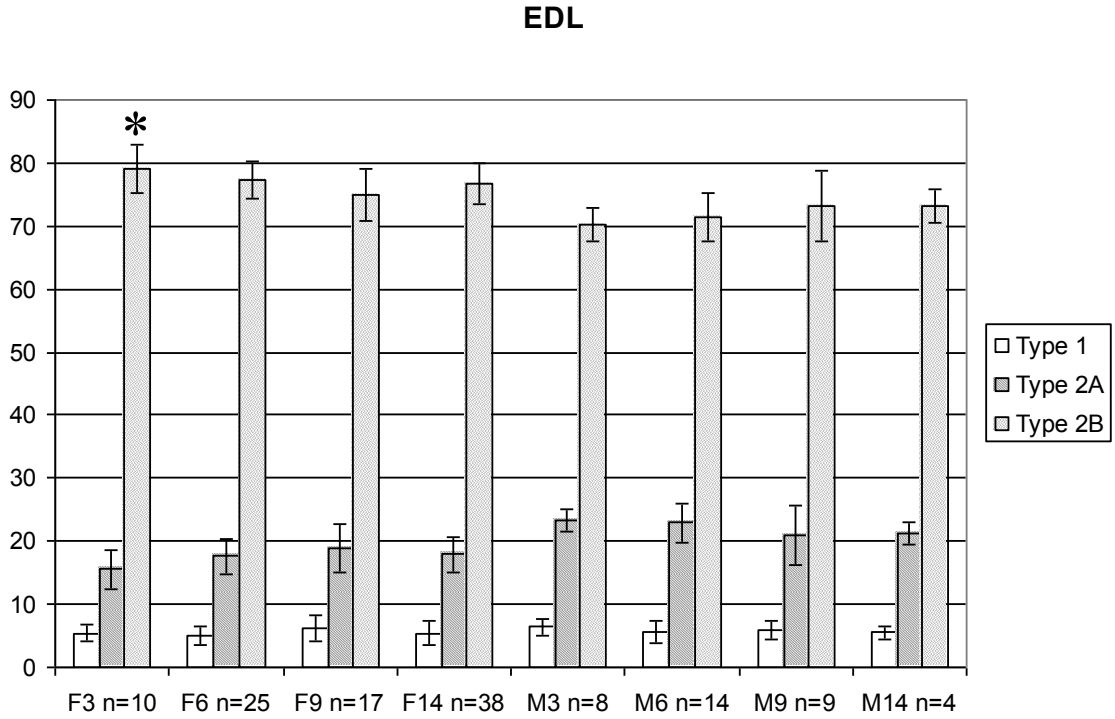

Fig. 5. Age differences in fibre type composition of the EDL muscle in female (F) and male (M) postnatal inbred Lewis strain rats in four age groups. Numerals on the x axis indicate age in months, n indicates the number of muscles analysed. Note that 3-month-old females, but not males, exhibit a significantly lower percentage of 2A and a higher percentage of 2B fibres 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 fibres among any age groups of either sex.

Note: The presented figures were published (Novák et al. 2010b).

5.1.3 Sex differences in fibre type composition

We did not find any significant difference in the content of type 1 and 2A fibres between male and female soleus muscles in any of the age groups (Figs. 4, 6). On the other hand, we found that the EDL muscles of the female rats contained significantly less 2A and more 2B fibres compared to the male rats, while there were no significant differences in the type 1 fibre proportion (Fig. 2B). Comparison of fibre type composition of the 3-month-old and older groups of male and female rats revealed different results in

the soleus and EDL muscles. While the significant difference in the content of type 1 and 2A fibres between the 3-month-old and older groups occurred both in male and female soleus muscles (Fig. 4), the EDL muscles of the 3-moth-old rats contained significantly less 2A and more 2B fibres compared to the older groups only in females, while no such tendency was observed in male rats (Figs 5, 7).

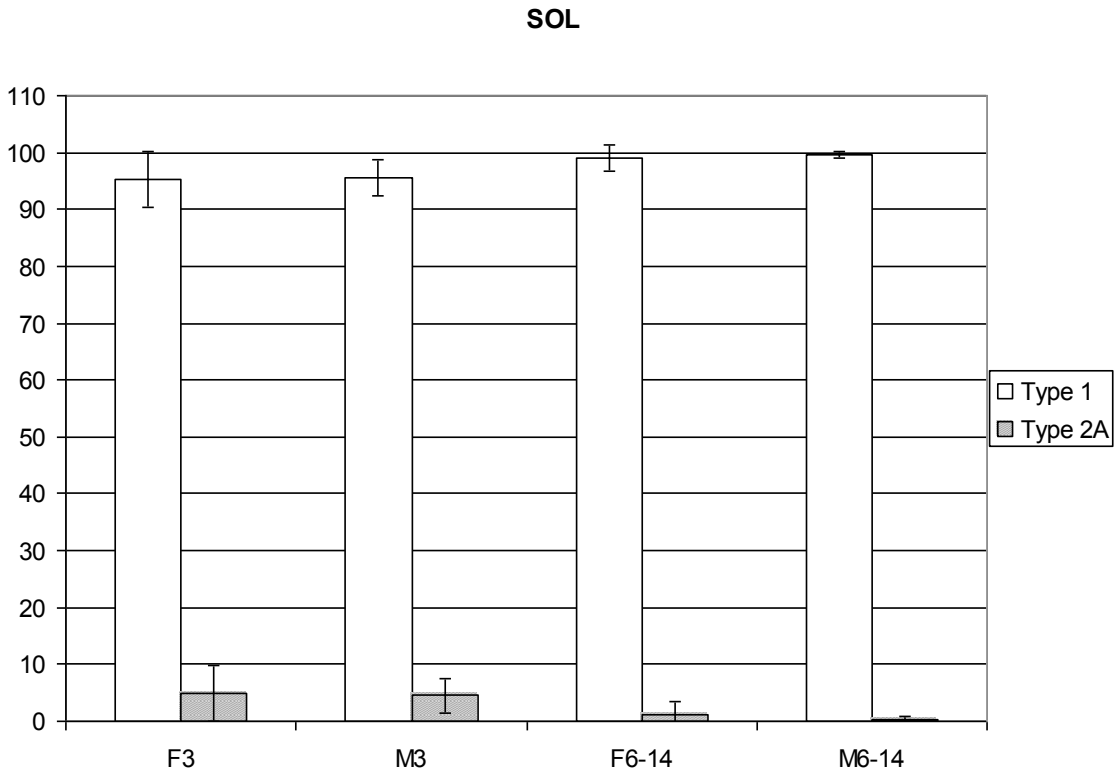

Fig. 6. Sex differences in the fibre type composition of the soleus (SOL) muscle between female (F) and male (M) inbred Lewis strain rats. Numerals on the x axis indicate age in months, the number of analysed muscles is the same as in Fig. 1A. Note that there are no significant differences between female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

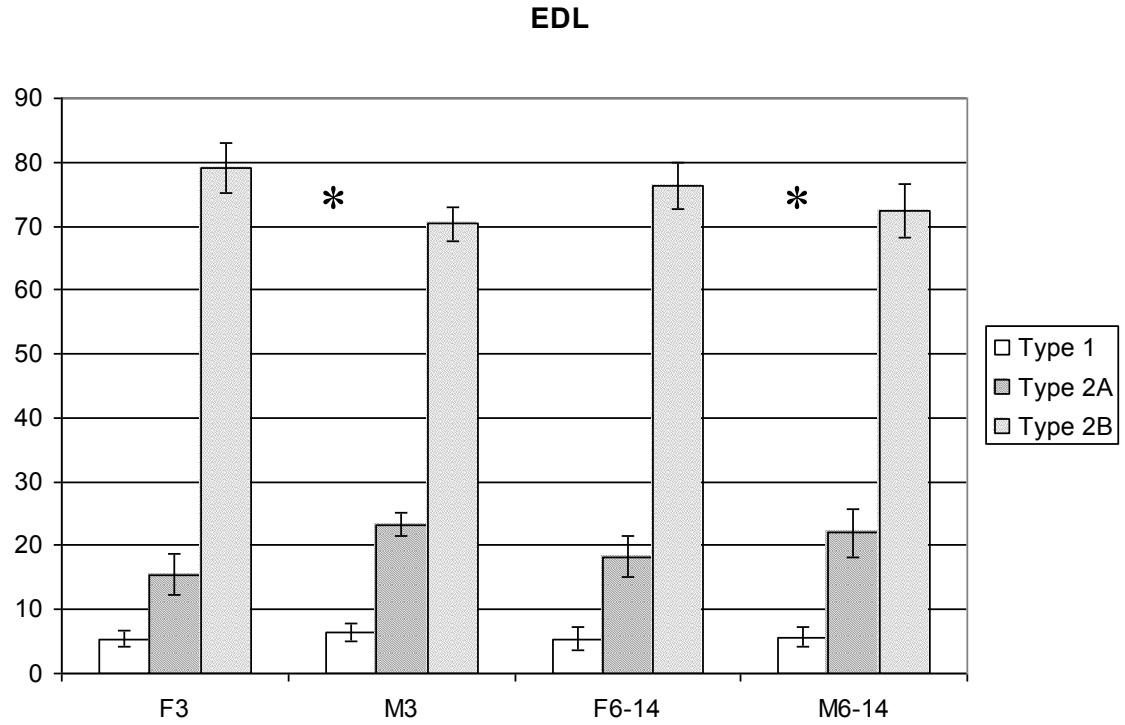

Fig. 7. Sex differences in the fibre type composition of the EDL muscle between female (F) and male (M) inbred Lewis strain rats. Numerals on the x axis indicate age in months, the number of analysed muscles is the same as in Fig. 2A. Note that there is a significant difference between female and male rats between 3-month and 6- to 14-month groups of female and male animals in the contents of 2A and 2B fibres (* indicates a significant difference between female and male animals, $p < 0.05$). The differences in the percentages of type 1 fibres are not significant.

Note: The presented figures were published (Novák et al. 2010b).

5.1.4 Strain differences in fibre type composition

Our data demonstrate that the soleus muscle of Lewis rats contains the highest percentage of type 1 fibres, comparable with literature data on WBN/Kob rats, but higher than Wistar, Sprague-Dawley, Fisher 344, Lister Hooded and SHR rats (Fig. 8, Supplement 1). The EDL muscles in all the examined strains contained a low number of type 1 fibres, varying between the lowest percentage in the Sprague-Dawley and the highest in the WBN/Kob rats (Fig. 9, Supplement 2). On the other hand, the highest

percentage of the fastest 2B fibres 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 less than 50 % of 2B fibres (Fig. 9, Supplement 2).

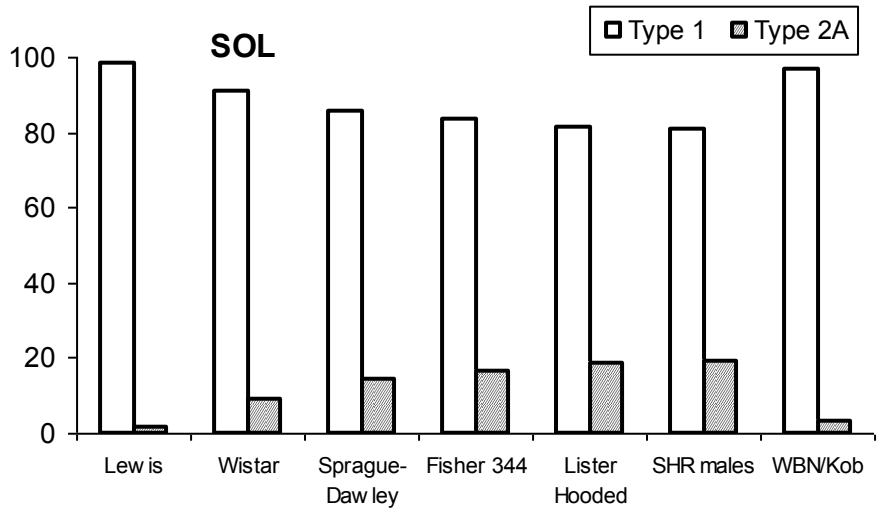

Fig. 8. Mean fibre type composition of the soleus (SOL) muscle of 4-month-old and older rats as summarised from the literature data on different rat strains (for further details see Supplement 1).

3B

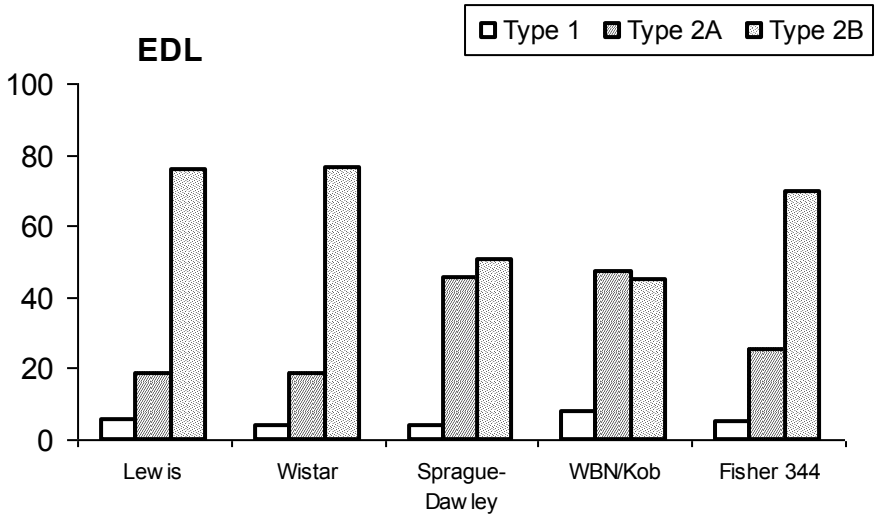

Fig. 9. Mean fibre type composition of the EDL muscle of 4-month-old and older rats as summarised from the literature data on different rat strains (for further details see Supplement 2).

Note: The presented figures were published (Novák et al. 2010b).

5.2 Effects of thyroid hormones on CaBPs and muscle fibre type composition

We have studied three CaBPs: sCSQ, PA and PhL. sCSQ was studied in the highest detail. Expression these CaBPs and the fibre type composition of fast and slow skeletal muscles of EU, HY and TH adult inbred female Lewis strain rats at protein levels were investigated in order to better understand, the chronic effects of thyroid hormone alterations.

Our pilot experiments (Novák *et al.* 2008, 2010a) in agreement with previous findings in the rat (Murphy *et al.* 2009) show that the protein levels for sCSQ are the highest in the fast EDL, intermediate in the soleus (Figs. 10, 11) and hardly detectable in the heart (not shown). The HY status decreases and the TH status increases the already high protein levels of sCSQ in the fast EDL (Figs. 10, 11). Neither the HY nor the TH status, however, has a significant effect on the "intermediate" levels of sCSQ found in the soleus muscle (Figs. 10, 11) and on the practically non-detectable levels in the heart (not shown).

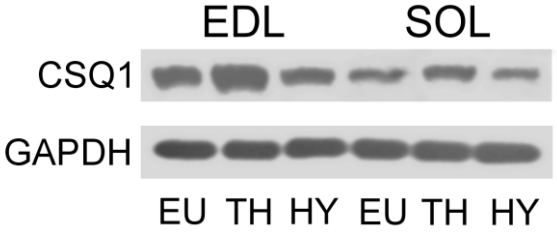

Fig. 10. An illustrative Western blot of sCSQ in the fast EDL and slow soleus (SOL) skeletal muscles of EU, TH (treated with T_3) and HY (treated with methimazole) 9- to 11-month-old inbred female Lewis strain rats. After separation of muscle proteins by SDS-PAGE, sCSQ was revealed on Western blots by specific monoclonal antibody. Corresponding amounts of GAPDH are shown in the bottom panel as loading control.

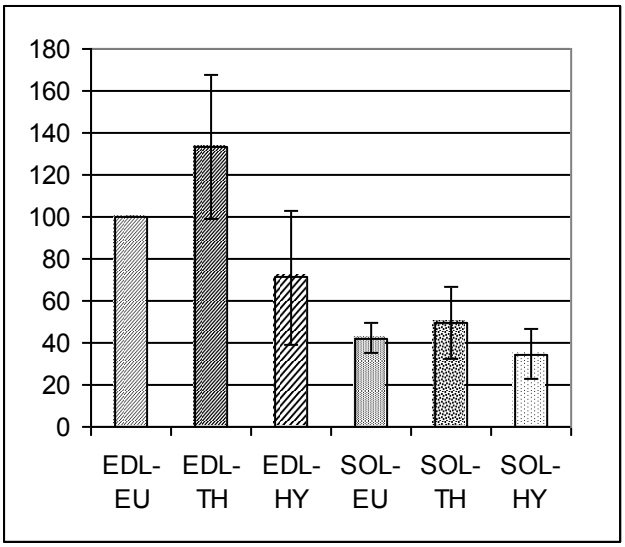

Fig. 11. The mean content of sCSQ in the fast EDL and slow soleus (SOL) skeletal muscles of EU, TH (treated with T_3) and HY (treated with methimazole) rats compared relatively to the content in the EDL of EU rats. Results are expressed in arbitrary units adjusted to GAPDH expression. The data represent average values (means ± S.D.) from 5 to 9 animals in each group; each value from an individual animal is based on 3 to 4 measurements (gels).

PA was expressed only in the fast EDL muscle, but not in the slow soleus muscle (Fig. 12) and in the heart (not shown), and its expression appeared decreased in the HY and TH compared to the EU rats (Fig. 13).

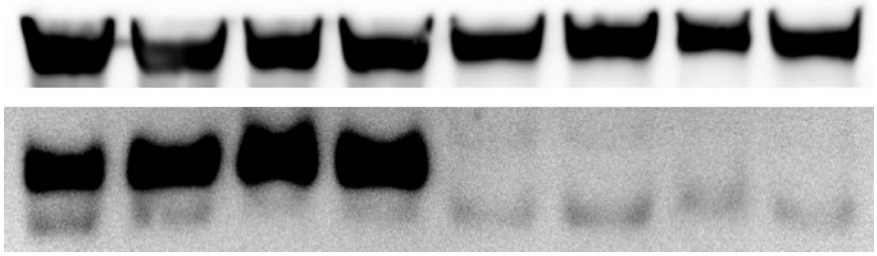

Fig. 12. PA is expressed in the EDL (four samples on the left), but not in the soleus (four samples on the right). After separation of muscle proteins by SDS-PAGE, PA was revealed on Western blots by specific monoclonal antibody (lower panel). The upper panel shows the expression of GAPDH.

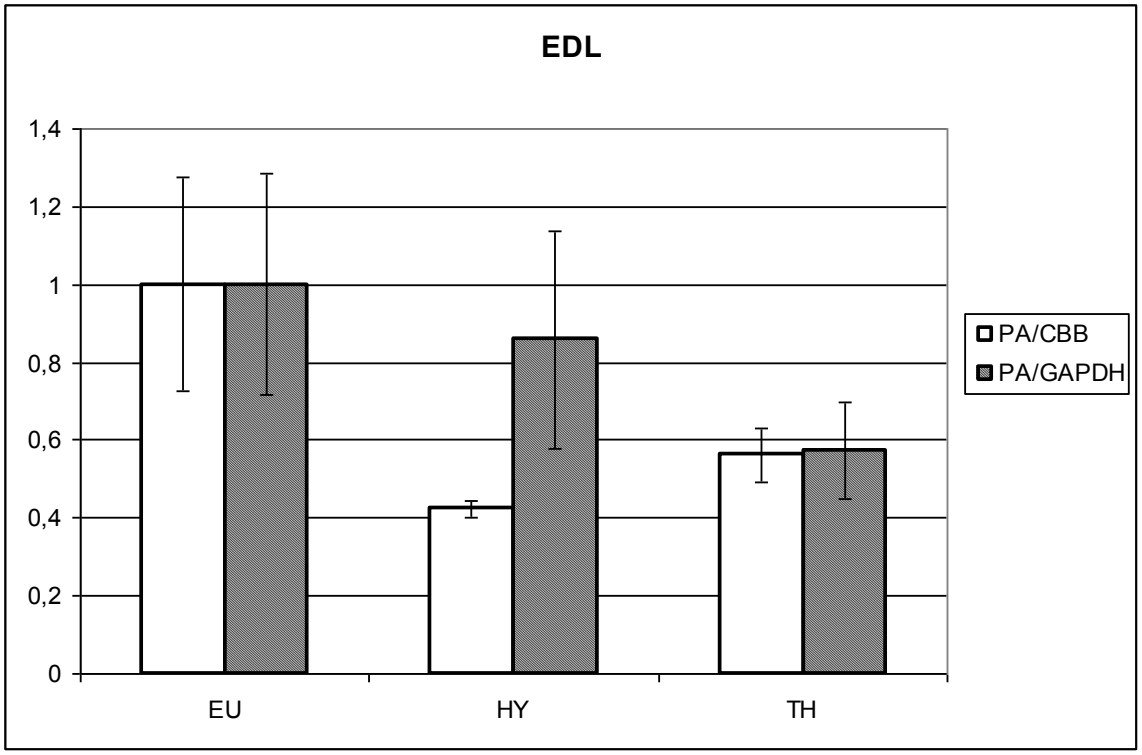

Fig. 13. Western blot analysis of PA in EDL muscles of EU, HY and TH rats. The expression of the analysed gene was normalised to the expression of either CBB or GAPDH. The data are presented as means ± S.E.M. (n=11).

PhL was expressed in its pentameric form (25 kDa) only in the soleus muscle and not in the fast EDL muscle, while in the ventricles, its monomeric form (5 kD) was expressed in great amount (Fig. 14). We found higher levels of PhL in the soleus muscle (Fig. 15), as well as in the ventricles (Fig.16) of the HY compared to the EU or TH rats.

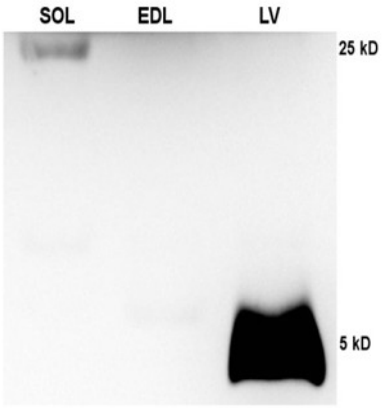

Fig. 14. The pentameric (25 kDa) form of PhL is expressed in the soleus (SOL), but not in the EDL muscle and the heart. On the other hand, in the left ventricle (LV), the monomeric (5 kDa) form is expressed in high amount, while it is not detected in skeletal muscles. After separation of muscle proteins by SDS-PAGE, PhL was revealed on Western blots by specific monoclonal antibody.

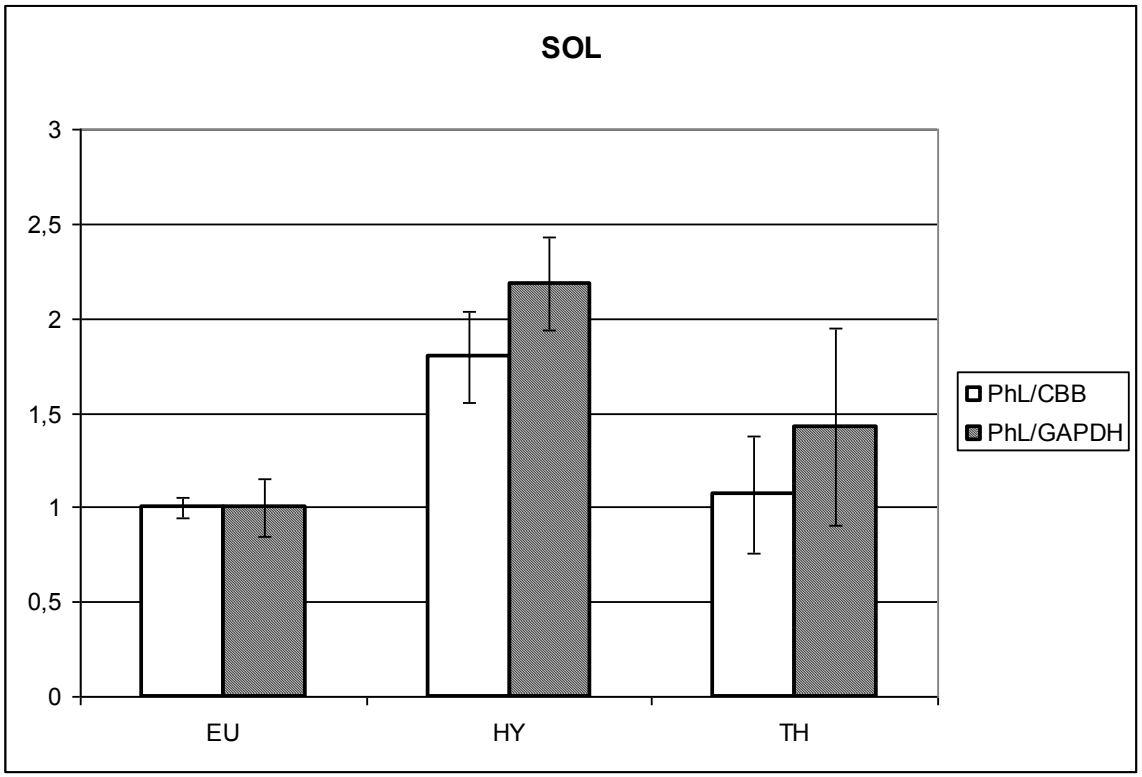

Fig. 15. Western blot analysis of PhL in soleus (SOL) muscles of EU, HY and TH rats. The expression of the analysed gene was normalised to the expression of either CBB or GAPDH. The data are presented as means ± S.E.M. (n=12).

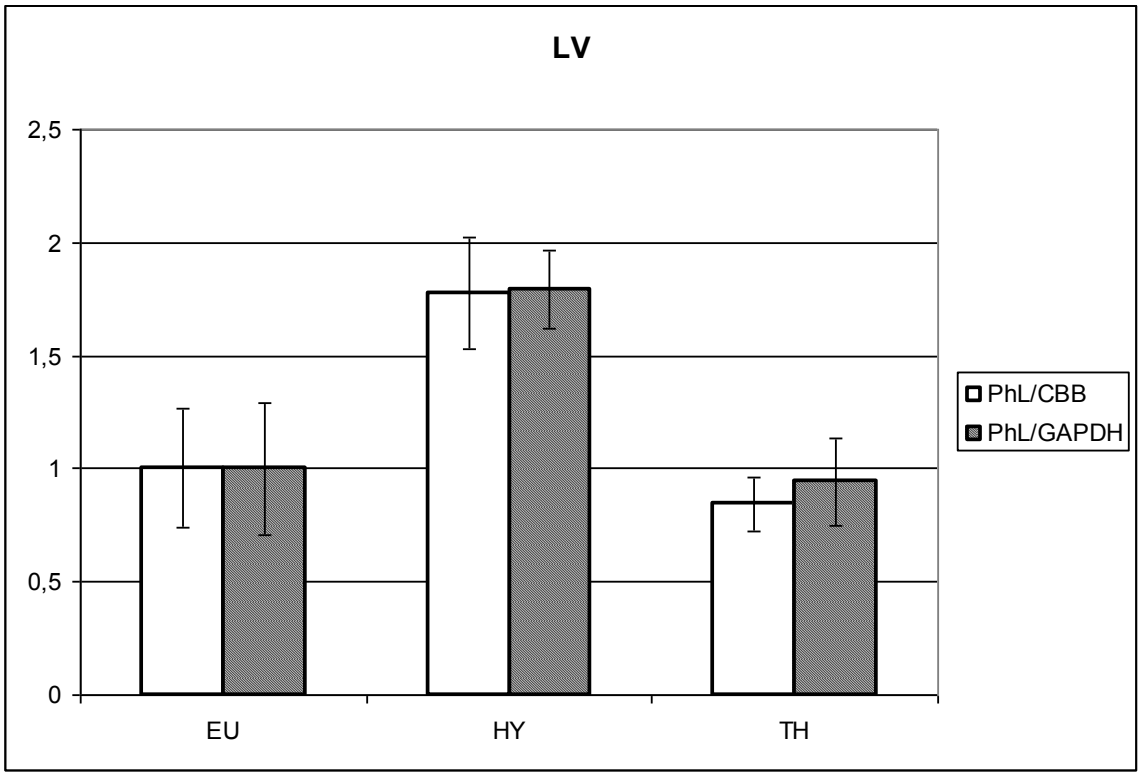

Fig. 16. Western blot analysis of PhL in left ventricles (LV) of EU, HY and TH rats. The expression of the analysed gene was normalised to the expression of either CBB or GAPDH. The data are presented as means ± S.E.M. (n=11).

The thyroid hormones also affect muscle fibre type composition (Table 1). The TH status significantly increased the proportion of 2A fibres in the soleus musle, while the HY status almost completely eliminated the 2A fibres from the soleus. In the EDL, the TH status significantly decreased the proportion of slow type 1 fibres, while the increase of 2B fibres was not significant. The HY status significantly decreased the percentage of the fastest 2B fibres and increased the percentage of the 2A fibres

THYROID STATUS	**HY**	**EU**	**TH**
EDL	*n=13*	*n=20*	*n=10*
1	9.7±5.3*	6.2±3.7	2.2±2.0***
2A	23.6±7.9**	15.8±3.8	15.9±4.6
2B	66.7±10.4***	78.0±5.2	82.0±5.3
THYROID STATUS	**HY**	**EU**	**TH**
SOL	*n=13*	*n=24*	*n=8*
1	99.6±1.2	99.2±1.2	65.7±7.2***
2A	0.4±1.2	0.8±1.2	34.3±7.2***

Table 1. Fibre type composition of the EDL and soleus (SOL) muscles as determined by mATPase reaction at pH 4.5 from 9- to 11-month-old EU, HY (treated with methimazole) and TH (treated with T_3) female inbred Lewis strain rats (the same set as analysed for the CSQ in Figs. 10, 11). The numerical proportions (%) of muscle fibre types were assessed by 2-D stereological methods using the principles of an unbiased counting frame and point counting by the C.A.S.T. Grid System (Olympus, Albertslund, Denmark) (Zacharova and Kubinova 1995, Zacharova et al. 1997, 1999, 2005). The data represent average values (means ± S.D.). n = number of muscles analysed. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ compared to the EU rats. The significance of the differences between the groups was evaluated by the Student`s t-test and/or Mann-Whitney test.

5.3 Effects of thyroid hormones on GPDH and anatomical parameters

5.3.1 Changes in GPDH activity

The measurements showed that the mean GPDH activity was significantly increased in TH and reduced in HY animals of both sexes (Table 2). The TH status increased GPDH activity 4.7 times in male and nearly 6.5 times in female rats compared to the respective EU controls. While the trend of enzyme activity changes in the TH and HY compared to the EU rats was the same in both sexes, in absolute numbers the EU female GPDH activity overwhelmed that of males by 23% and that of the TH females by even 45%. In the HY status, GPDH activity was significantly reduced to similarly low values in both sexes with no significant difference between male and female rats, but due to the difference in the EU levels, GPDH activity in the female was reduced by 62%, while in the male rats by 48% of the respective EU levels.

Sex	**Thyroid status**	**GPDH activity**	**n**
Male	EU	7.7 ± 2.1	15
	TH	$35.9 \pm 12.3^{***}$	9
	HY	$4.0 \pm 1.2^{***}$	9
Female	EU	$10.0 \pm 1.5^{\#}$	14
	TH	$64.3 \pm 18.8^{***\#\#\#}$	15
	HY	$3.8 \pm 1.0^{***}$	10

The values represent means ± SD, $^{***}p \leq 0.001$ compared to the EU counterparts, $^{\#}p \leq 0.05$, $^{\#\#\#}p \leq 0.001$ compared male to female rats, n = numbers of animals.

Table 2. GPDH activity (measured spectrophotometrically as GPDH:cyt *c* in nmol/min/mg protein) of male and female inbred Lewis rat liver mitochondria with the EU, TH (rats treated with T_3) and HY (rats treated with methimazole) status. All measurements were performed by Dr. H. Rauchová.

Note: The presented table was published (Rauchová et al. 2010).

5.3.2 Changes in the GPDH protein amount

GPDH expression, shown as the enzyme protein amount detected by specific anti-GPDH antibody by Western blotting, was significantly increased in the TH and decreased in the HY status in both sexes (Fig. 17A,B). The content of GPDH increased about 3 times in the TH animals and decreased to approximately one third both in the female and male HY rats compared to the respective EU controls. Similarly to enzyme activity, the GPDH amount in the EU and TH males was lower by 23% and 25%, respectively, compared to the female rats. Correspondingly, the GPDH amount in the HY male rats was 16% lower when compared to the female rats. However, due to the inherent semiquantitative nature of Western blot, the differences in the EU and HY animals did not reach statistical significance.

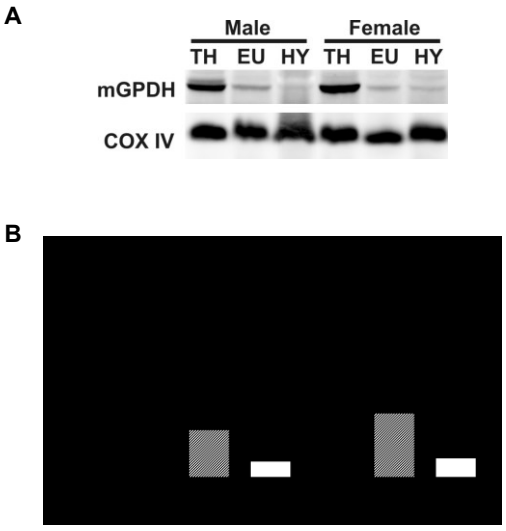

Fig. 17 (A) An illustrative Western blot of liver mitochondrial GPDH in EU, HY (treated with methimazole) and TH (treated with T_3) male (M) and female (F) inbred Lewis rats. Corresponding amounts of cytochrome *c* oxidase (COX IV) are shown in the bottom panel as loading control. (B) The mean content (mean ± SD) of GPDH normalised to COX IV

(arbitrary units) compared relatively to the content in female EU rats. The data represent average values from four animals in each group; each value from an individual animal is based on 3 to 7 measurements (gels). $^{*}p\leq0.05$, $^{**}p\leq0.01$, $^{***}p\leq0.001$ compared to the EU rats, $^{\#}p\leq0.05$, compared to female rats. The analysis was performed by Dr. T. Mráček.

Note: The presented figures were published (Rauchová et al. 2010).

5.3.3 Changes of levels of thyroid hormones and anatomical parameters

We measured levels of thyroid hormones and anatomical parameters in all experiments dealing with alterations of the thyroid status. Here I present results from the paper by Rauchová et al. (2010), but all the other measurements used for the analysis of changes of fibre type composition and CaBPs fully comply with the presented ones. We found that the level of tT_3 was reduced by two thirds (Fig. 18A) and that of T_4 to one tenth (not shown) in the HY status, while the TH status increased tT_3 levels approximately 5 times (Fig. 18A), in contrast to tT_4, which was reduced almost to the HY level due to the negative feedback control caused by the very high levels of T_3 (not shown). Hypothyroidism led to an increase of absolute and relative (thyroid gland weight/body weight, mg/g) thyroid gland weights and to a decrease of absolute and relative (heart weight/body weight, mg/g) heart weight, while hyperthyroidism led to opposite changes (Fig. 18B, C).

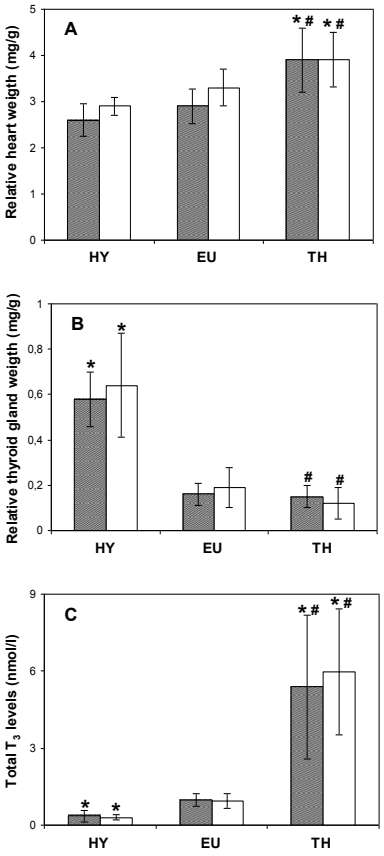

Fig. 18 Absolute serum levels of tT_3 (A) as well as relative thyroid gland (B) and heart (C) weights of EU, HY (treated with methimazole) and TH (treated with T_3)male (hatched columns) and female (blank columns) inbred Lewis strain rats used for the GPDH measurements (for number of rats see Table 1)**.** Note that there are no statistically significant differences in the above parameters between male and female rats. The values represent mean ± S.D., *significant difference ($p \leq 0.05$) against EU, $^{\#}$significant difference ($p \leq 0.05$) between HY and TH rats.

Note: The presented figure was published (Rauchová et al. 2010).

6 DISCUSSION

6.1 Fibre type composition

Our results on the inbred Lewis strain rats confirmed that i) the fibre 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 fibres and more 2A fibres compared to older animals, iii) there is a sex difference in the proportion of 2A and 2B fibre types in the EDL muscle and iv) the fibre type composition of inbred Lewis strain rats differs from literature data of other routinely used rat strains.

6.1.1 Justification of mATPase reaction for fibre typing

As the great majority of studies analysing fibre 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, a previous study (Soukup et al. 2009) showed no significant difference in the percentage of type 1 and type 2A fibres in the soleus and EDL muscles based on mATPase reaction compared to immunocytochemical determination using specific monoclonal antibodies against type 1 and 2A fibres. The undisputable advantage of immunoreactions enabling separate recognition of 2X/D fibres does not bring any gain when comparing with literature data based on the division into three fibre types (1, 2A and 2B) as shown in the Supplements 1 and 2..

6.1.2 Fibre type composition and individual variability

Our present results for Lewis rats correspond very well with previously published data (Soukup et al. 2002, 2009, Zacharova et al. 2005). In the soleus muscle, the percentage of 2A fibres varied between zero and up to about 13 %, although some fibres, determined as 2A fibres, apparently bore a resemblance to hybrid 2C fibres. It is well-known that the soleus hybrid fibres exhibit physiological characteristics between slow type 1 and fast 2A fibres (for review see Pette and Staron 2001, Stephenson 2006). Although the 2A fibres are capable of faster contraction, they are similarly as the type 1 fibres 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 fibres, a medium number of fast 2A and a majority of the fastest 2B fibres. Although individual EDL muscles in Lewis rats exhibit different proportions of 2A and 2B fibres 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 fibre type differences among individual rats was already recognised previously (Hall-Craggs et al. 1983, Li et al. 1996, Soukup et al. 2009) and it can, however, significantly affect the fibre type percentage in studies analysing 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 fibres in the muscle, which is not the case in many other studies. An estimate of fibre 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 fibres, while the latter predominantly from 2A fibres supplemented by type 1 fibres (e.g. analysis of the red portion would thus increase the percentage of 2A against 2B fibres).

6.1.3 Age differences in fibre type composition

There are many studies analysing development of the soleus and less of the EDL muscle during the early postnatal period, but only few of them describe fibre 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 analysing the soleus all describe significant increase of slow type 1 and decrease of type 2A fibres during the first two postnatal months followed by minor changes during the 3rd and 4th months. Our results showed that both the male and female Lewis rats in the 3-month-old group still contained in the soleus muscle a lower percentage of type 1 fibres compared to the older rats. The literature data on Wistar rats (Supplement 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) found difference between 3- to 7- and 20- to 25-month-old Wistar rats that contained about 92 and 96 % of type 1 fibres, 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 fibres on the expense of type 1 and 2B fibres 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 fibre 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.

6.1.4 Sex differences in fibre type composition

Our data did not reveal any significant sex difference in the composition of the soleus muscle, although the adult males contained rather higher percentages of type 1 (and lower percentages of 2A) fibres than the females as more male than female soleus muscles were solely composed of slow type 1 and 1C fibres. It also appeared that soleus muscles in the males achieved their "slow" composition earlier than in the 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. the Wistar young females contained less type 1 fibres 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. Supplement 2). Although the most evident growth differences between the 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 fibre type composition either in young or older animals (Supplement 1). On the other hand, we found a significant difference in the EDL muscle between the female and male inbred Lewis rats, as the females contained more of faster 2B and less of 2A fibres compared to the male EDL muscles (Fig. 7). The latter difference also appeared from the comparison of literature data on the Wistar rats (cf. Supplement 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 fibres 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).

6.1.5 Strain differences in fibre type composition

Comparison of fibre 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 fibres, followed by WBN/Kob, Wistar and Sprague-Dawley, Fisher 344, Lister Hooded and SHR rats (Supplement 1, Fig. 8). Furthermore, the inbred Lewis rats attain the very high percentage of type 1 fibres in the soleus muscle earlier than the other strains. It can be related to their higher natural levels of serum T_4 (http://www.harlan.com/research_models_and_services). This fact was demonstrated experimentally, as TH rats achieved adult soleus composition earlier than EU and HY rats (Vadászová-Soukup and Soukup 2007). In the EDL muscle, the highest percentage of the fastest 2B fibres (and the lowest of 2A fibres) was exhibited by the Lewis, Wistar and Fisher rats, while the Sprague-Dawley and WBN/Kob contained an almost equal percentage of 2B and 2A fibres (Supplement 2, Fig. 9).

It was shown that the soleus muscle of the SHR rats contains a three times greater proportion of fast fibres 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 fibres 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 the SHR and Lewis rats as the percentage of the type 1 fibres in soleus muscles ranges from about 80 % in the SHR to almost 99 % in the Lewis rats. Similarly, the 25-percentage-point difference in the content of type 2B fibres in the EDL between the Sprague-Dawley or WBN/Kob (about 50 %) and the Lewis or Wistar rats (about 75 %) seems to be high enough to have physiological consequences. Our results show that regarding soleus fibre type composition, the Lewis and WBN/Kob rats form a group of "very slow" strains, while the Sprague-Dawley, Fisher 344, Lister Hooded and SHR correspond to the "relatively faster" strains, with the Wistar rats in between these two groups. Regarding the EDL, however, the Lewis and Wistar rats form the "fast" group, while the Sprague-Dawley and WBN/Kob represent the "relatively slower" strains, the Fisher 344 being in between these groups. It seems that the muscle fibre 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.

6.2 Effects of thyroid hormones on CaBPs and muscle fibre type composition

Experimental studies analysing effects of thyroid hormone levels on CaBPs expression focused mainly on cardiac muscle apparently due to the profound impact of thyroid hormones on the heart function mediated by regulating the transcription of genes for calcium transporter proteins of the sarcolemma and the SR and for specific myofibrillar proteins (for review see Dillmann 1990). Measurements of SERCA2, NCX and PhL in the rat heart showed that atria exhibit a greater change in the protein content than ventricles in response to T_3 exposure (Shenoy et al. 2001). Much less is known about regulation of Ca^{2+} transport systems due to altered concentrations of thyroid

hormones in skeletal muscle (e. g. Simonides and van Hardeveld 1985, Connelly et al. 1994). It was found that the TH status increases and the HY status decreases protein and mRNA levels of RyR and SERCA after acute 4- or 8-day treatment in rabbits (Arai et al. 1991) and mRNA of RyR1 and 2, NCX and type 2 inositol-1, 4, 5-triphosphate receptors after chronic alteration in rats (Hudecová et al. 2004).

Studies related to CSQ changes in animals with the altered thyroid status are exceptional. Arai et al. (1991) found that the acute changes of the thyroid status for 4 and 8 days in the rabbit soleus might suggest that hyperthyroidism increased expression of sCSQ compared to cCSQ, while hypothyroidism had rather the opposite effect. The same authors reported that cCSQ expression in rabbit ventricles was only slightly increased after both treatments and their results also implied possible differences in cCSQ expression between ventricles and atria.

To my knowledge, PA in muscles of animals with altered thyroid states was analysed only by Müntener et al. (1987), who investigated EDL, soleus, and gastrocnemius muscles of rats and found that PA distribution and concentration were largely unaffected in all thyroid states after a 4 week treatment.

The results for PhL in the heart are in agreement with published data (for review see Carr and Kranias 2002). Little is known about PhL in skeletal muscles. Jiang et al. (2004) reported downregulation of PhL in TH rabbit soleus muscles after daily intramuscular T_4 injections for 7 days, while the chronic TH status suggests no change or only a small increase in PhL expression. On the other hand, our results show an obvious increase of PhL expression both in the soleus and left ventricles in the HY compared to EU rats. As the HY status slows down contractile characteristics and increases slow MHC 1/beta isoform, our finding is in agreement with PhL inhibitory effects on SERCA that pumps calcium ions into the lumen of the SR, initiating and speeding muscle relaxation (Berchtold et al. 2000, Traaseth et al. 2008).

Our preliminary comparison of fibre type and CSQ level changes indicates that the observed increase of the sCSQ level can be caused by the increase of the fastest 2B fibres in the TH status (where they form more than 80% of all the fibres), while the decreased level of sCSQ in the HY rats can result from a decline in proportion of these fibres (Table 1 EDL). No changes of the sCSQ levels in the soleus can be explained by

the lack of the fastest 2B fibres and the significant increase of 2A fibres in the TH soleus has surprisingly only small and nonsignificant effect on the increase of the sCSQ level. Similarly, Murphy et al. (2009) found more than a 3x higher content of sCSQ in the EDL type 2 fibres compared to the soleus type 1 fibres and they also presumed the existence of differences in sCSQ levels between subgroups of the fast type 2 fibres. Our results thus suggest that the observed changes in expression of sCSQ in rat muscles resulting from chronic alteration of the thyroid hormone levels are more likely a part of complex fibre type changes induced by thyroid hormones and revealed by the switch of MyHC isoforms and muscle fibre types. The effect of T_3 levels on CSQ gene transcription would be thus regulated co-ordinately with other proteins rebuilt during muscle fibre type transformation and the expression of sCSQ apparently proceeds in a fibre type-specific manner. The final answer can be, however, obtained only by a single fibre analysis of the MyHC isoforms and sCSQ content.

Experiments on CaBPs relating to thyroid hormone levels suggest that SR calcium storage capacity (CSQ) is less affected than the calcium release (RyRs) and uptake (SERCA, NCX) and that minor changes observed in muscles of rats with altered hormone levels are probably related to complex changes taking part during muscle fibre type transformation.

6.3 Effects of thyroid hormones on GPDH and anatomical parameters

6.3.1 Changes in GPDH activity

The results confirm previous data (Rauchová et al. 2004) obtained on female rat livers reflecting the level of thyroid hormones in the organism and demonstrate the same changes in male rats. The data show that chronic alteration of T_3 affects both the activity and protein amount of GPDH similarly in the male and female rats, although the GPDH activity of the EU adult females was significantly higher (by about 25%) than that of the age-matched males. The significant difference in GPDH activity between the EU males and females was also observed in adult (80-day-old) and immature (20-day-old) Wistar rats (Coleoni and Cherubini 1989). Similarly, Alfadda et al. (2004) found basal (EU) female mice liver GPDH significantly higher (by a margin of about 30%), but in other tissues (such as brain, kidney, muscle or brown adipose tissue) differences between the

males and females were not significant. Although the chronic effect of the HY and TH states was evident in both sexes, the TH status lead to a higher increase and the HY status to a higher decrease of GPDH activity in the females compared to the males. Similarly, Coleoni and Cherubini (1989) described markedly higher T_3-induced GPDH activity after a single high dose of T_3 in the female rats. These observations suggest that the females are more sensitive to thyroid hormone alterations than the male rats. It is generally accepted that testosterone has a protective role against thyreotoxicosis, while estrogen supports the development of the disease. Interestingly, the impairment of the thyroid gland in humans affects about 5-7% of population and the ratio between women and men among these patients is 6-8:1 (Límanová 2009).

6.3.2 Changes in the GPDH protein amount

Sellinger and Lee (1964) first suggested (according to their experiments with puromycin and actinomycin) that an increase in GPDH activity after a single T_3 injection must also involve a synthesis of new enzyme molecules. Later, Oppenheimer et al. (1977) showed in acute experiments that there is a good correlation among the T_3 serum concentrations, specific nuclear T_3 receptor site occupancy and increased activity of rat liver GPDH after a single dose of T_3. Seitz and coworkers demonstrated fast (during 4-6 hours) and high induction of GPDH mRNA in rat liver after a single intraperitoneal injection of T_3 (Dümmler et al. 1996, Müller and Seitz 1994). The data correspond well to recent experiments where the kinetics of the T_3 level in blood, mRNA level, protein content and enzyme activity of liver GPDH were followed after either one or three doses of T_3 (Mráček et al. 2005). We did not find any data on GPDH expression after chronic changes of the thyroid status. Our present data, however, show that chronic administration of T_3 increases GPDH enzyme activity as well as protein synthesis in similar proportions, suggesting that the increase in activity depends on the increase of the protein amount.

6.3.3 Changes of levels of thyroid hormones and anatomical parameters

The thyroid hormone level and anatomical parameter changes demonstrate the efficacy and reliability of our protocol for inducing hypo- and hyperthyroidism. Together

with the determination of rat liver GPDH activity, these parameters appear as convenient markers of the thyroid status in chronic experiments. Furthermore, all characteristics measured in the presented group of animals fit well with the results obtained earlier on different samples (Rauchová et al. 2004, Soukup et al. 2001).

6.4 Conclusion.

1. Our results revealed substantial individual variability in muscle fibre 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 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 "specialised" in respect to skeletal muscle composition, as their soleus is the slowest and their EDL is the fastest among the compared rat strains.

2. The experiments on calcium binding proteins showed that calsequestrin, parvalbumin and phospholamban are to a certain extent affected by altered thyroid hormone levels and that these changes can be related to fiber type changes resulting from the alteration of the thyroid status. These results, however, does not rule out an involvement of these proteins in thyroid hormone-related alterations of the calcium homeostasis.

3. Long lasting chronic experiments showed a similar effect of altered thyroid hormone levels on the GPDH activity and protein content in female and male rats, although in absolute numbers female EU rats had both the higher enzyme activity and content than their male counterparts. The measurements confirmed that the determination of the GPDH activity and/or amount can serve as an additional criterion for the evaluation of the thyroid hormone status.

4. Our experiments showed that the muscle fibre type composition is not fixed as it shows individual, sex and strain differences. Furthermore, it can be changed by external factors, e. g. by thyroid hormones that can affect expression of not only MHCs, but also of CaBPs. The changes of both of the components playing a decisive role in muscle

contraction are a prerequisite for the change of complete physiological performance of individual fibre types.

7 SUMMARY

The goals of this study were to analyse the contribution of individual, age, sex and strain differences to the variability of muscle fibre type composition, to investigate chronic effects of the thyroid status alterations on expression of calcium binding proteins (calsequestrin, parvalbumin and phospholamban) and to demonstrate reliable methods for evaluating the altered thyroid status. The experiments were performed on fast extensor digitorum longus (EDL) and slow soleus muscles of inbred Lewis strain rats.

The analysis of muscle fibre type composition confirmed that i) the fibre 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 fibres and more 2A fibres compared to older animals, iii) there is a sex difference in the proportion of 2A and 2B fibre types in the EDL muscle and iv) the fibre type composition of inbred Lewis strain rats differs from literature data of other routinely used rat strains.

The experiments on calcium binding proteins showed that calsequestrin, parvalbumin and phospholamban are to a certain extent affected by altered thyroid hormone levels and that these changes can be related to fibre type changes resulting from the alteration of the thyroid status. These results, however, do not rule out an involvement of these proteins in thyroid hormone-related alterations of the calcium homeostasis.

Long lasting changes of the thyroid status have an effect on the GPDH activity and protein content and on heart and thyroid gland weights of female and male rats. These findings confirmed that the determination of the GPDH activity and/or amount, as well as analysis of selected anatomical parameters, can serve as an additional criterion for the evaluation of the thyroid hormone status in chronic experiments.

In conclusion, the individual, sex and interstrain differences show that the muscle fibre type composition is not fixed. Furthermore, it can be changed by external factors, e. g. by thyroid hormones that can affect expression of not only myosin heavy chains, but partly also of calcium binding proteins. The changes of both of these components playing a decisive role in muscle contraction and relaxation are a prerequisite for the change of complete physiological performance of individual fibre types.

8 SOUHRN

Cílem této práce byla analýza podílu individuálních, věkových, pohlavních a mezikmenových rozdílů na variabilitu složení svalových vláken, studium chronických účinků změněného tyroidního stavu na expresi proteinů vážících vápník (kalsekvestrinu, parvalbuminu and fosfolambanu) a demonstrace spolehlivých metod hodnocení změněného tyroidního stavu. Experimenty byly prováděny na rychlých (extensor digitorum longus, EDL) a pomalých svalech (soleus) potkanů inbredního kmene Lewis.

Analýza složení svalových vláken potvrdila, že i) složení svalových vláken se nemění po čtvrtém měsíci věku, ii) soleus tříměsíčních potkanů ale obsahuje signifikantně méně vláken typu 1 a více vláken typu 2A ve srovnání se staršími zvířaty, iii) existuje pohlavní rozdíl v podílu vláken typu 2A a 2B v EDL a iv) složení svalových vláken potkanů inbredního kmene Lewis se liší od hodnot pro ostatní běžně užívané kmeny potkanů uváděných v literatuře.

Experimenty týkající se proteinů vážících vápník ukázaly, že kalsekvestrin, parvalbumin a fosfolamban jsou do určité míry ovlivněny změnami hladin tyroidních hormonů a že tyto změny mohou souviset se změnami typů svalových vláken způsobených alteracemi tyroidního stavu. Typo výsledky však nevylučují podíl těchto proteinů na změnách homeostázy vápníku souvisejících se změnami tyroidního stavu.

Dlouhodobé změny tyroidního stavu ovlivňují aktivitu a obsah GPDH a hmotnost srdce a štítné žlázy u samců i samic. Typo výsledky potvrdily, že stanovení aktivity nebo množství GPDH, stejně jako měření vybraných anatomických parametrů, může sloužit jako kritérium pro určení tyroidního stavu v dlouhodobých experimentech.

Individuální, pohlavní a mezikmenové rozdíly ukazují, že složení svalových vláken není neměnné. To může být navíc ovlivněno působením vnějších faktorů, např. tyroidních hormonů, které mohou ovlivnit expresi nejen těžkých řetězců myozinu, ale i proteinů vážících vápník. Změny obou těchto komponent hrajících rozhodující roli ve svalové kontrakci jsou nezbytným předpokladem pro změnu celkového fyziologického výkonu jednotlivých typů svalových vláken.

9 REFERENCES

ABOUDRAR S, SEMPORE B, KOUBI H, DECHAUD H, DESPLANCHES D: Effects of adrenalectomy or RU-486 on rat muscle fiber during hindlimb suspension. J Appl Physiol 75: 2767-2773, 1993.

ALFADDA A, DOSSANTOS RA, STEPANYAN Z, MARRIF H, SILVA JE: Mice with deletion of the mitochondrial glycerol-3-phosphate dehydrogenase gene exhibit a thrifty phenotype: effect of gender. Am J Physiol Regul Integr Comp Physiol 287: R147-R156, 2004.

ALMEIDA-SILVEIRA MI, PÉROT C, POUSSON M, GOUBEL F: Effects of stretch-shortening cycle training on mechanical properties and fibre type transition in the rat soleus muscle. Pflugers Arch 427: 289-294, 1994.

ANSVED T: Effects of immobilization on the rat soleus muscle in relation to age. Acta Physiol Scand 154: 291-302, 1995.

ARAI M, OTSU K, MACLENNAN DH, ALPERT NR, PERIASAMY M: Effect of thyroid hormone on the expression of mRNA encoding sarcoplasmic reticulum proteins. Circulation Research 69: 266-276, 1991.

ARIANO MA, ARMSTRONG RB, EDGERTON VR: Hindlimb muscle fiber populations of five mammals. J Histochem Cytochem 21: 51-55, 1973.

ARMSTRONG RB, PHELPS RO: Muscle fiber type composition of the rat hindlimb. Am J Anat 171: 259-272, 1984.

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

ATRAKCHI A, GRAY SD, CARLSEN RC: Development of soleus muscles in SHR: relationship of muscle deficits to rise in blood pressure. Am J Physiol 267: C827-C835, 1994.

BÁRÁNY M: ATPase activity of myosin correlated with speed of muscle shortening. J. Gen. Physiol. 50: 197-218, 1967.

BEARD NA, LAVER DR, DULHUNTY AF: Calsequestrin and the calcium release channel of skeletal and cardiac muscle. Prog Biophys Mol Biol 85: 33-69, 2004.

BEARD NA, WEI L, DULHUNTY AF: Ca(2+) signaling in striated muscle: the elusive roles of triadin, junctin, and calsequestrin. Eur Biophys J 39: 27-36, 2009.

BERCHTOLD, MW, BRINKMEIER H, MÜNTENER M: Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. Physiol Rev 80: 1215-1265, 2000.

BERRIDGE MJ, BOOTMAN MD, RODERICK HL: Calcium signalling: dynamics, homeostasis and remodelling. Nat Rev Mol Cell Biol 4: 517-29, 2003.

BIGARD AX, LIENHARD F, MERINO D, SERRURIER B, GUEZENNEC CY: Effects of growth hormone on rat skeletal muscle after hindlimb suspension. Eur J Appl Physiol 69: 337-343, 1994.

BROOKE MH, KAISER KK : Muscle fiber types :How many and what kind? Arch. Neurol. (Chic.) 25: 360-366, 1970.

CAIOZZO VJ, BAKER MJ, McCUE SA, BALDWIN KM: Single-fiber and whole muscle analyses of MHC isoform plasticity : interaction between T3 and unloading. Am J Physiol 273: C944-C952, 1997.

CANON F, BIGARD AX, MERINO D, LIENHARD F, GUEZENNEC CY: Effects of chronic low frequency stimulatin on structural and metabolic properties of hindlimb suspended rat soleus muscle. Eur J Appl Physiol 70: 528-535, 1995.

CARAFOLI E, SANTELLA L, BRANCA D, BRINI M: Generation, control, and processing of cellular calcium signals. Crit Rev Biochem Mol Biol 36: 107-260, 2001.

CARAFOLI E: Calcium signaling: a tale for all seasons. Proc Natl Acad Sci U S A 99: 1115-22, 2002.

CARR AN, KRANIAS EG: Thyroid hormone regulation of calcium cycling proteins. Thyroid 12: 453-7, 2002.

CHAMBERLAIN S, LEWIS DM: Contractile characteristics and innervation ratio of rat soleus motor units. J Physiol 412: 1-21, 1989.

CLAPHAM DE: Calcium signaling. Cell 131: 1047-58, 2007.

COLEONI AH, CHERUBINI O: Sex-related differences in the activity of liver mitochondrial α-glycerophosphate dehydrogenase in the rat. Acta Physiol Pharmacol Latinoam 39: 245-253, 1989.

CONNELLY TJ, EL-HAYEK R, SUKHAREVA M, CORONADO R: L-thyroxine activates the intracellular Ca2+ release channel of skeletal muscle sarcoplasmic reticulum. Biochem Mol Biol Int 32: 441-8, 1994.

D´ALBIS A, COUTEAUX R, JANMOT C, MIRA JC The same myosin isoforms are found in the female and male sexually dimorphic levator ani muscle of the rat, but their postnatal transitions are not synchronous. FEBS Lett 278: 41-4, 1991.

D'ALBIS A, BUTLER-BROWNE G: The hormonal control of myosin isoform expression in skeletal muscle of mammals: a review. Basic Appl Myol 3: 7-16, 1993.

DESPLANCHES D, MAYET MH, SEMPORE B, FLANDROIS R: Structural and functional responses to prolonged hindlimb suspension in rat muscle. J Appl Physiol 63: 558-63, 1987.

DILLMANN WH: Biochemical basis of thyroid hormone action in the heart. Am J Med 88: 626-30, 1990

DRÁBER P, LAGUNOWICH LA, DRÁBEROVÁ E, VIKLICKÝ V, DAMJANOV I: Heterogeneity of tubulin epitopes in mouse fetal tissues. Histochemistry 89: 485-492, 1988.

DULHUNTY AF: Excitation-contraction coupling from the 1950s into the new millennium. Clin Exp Pharmacol Physiol 33: 763-72, 2006.

DÜMMLER K, MÜLLER S, SEITZ HJ: Regulation of adenine nucleotide translocase and glycerol-3-phosphate dehydrogenase expression by thyroid hormones in different rat tissues. Biochem J 317: 913-918, 1996.

EGGINTON S: Numerical and areal density estimates of fibre type composition in a skeletal muscle (rat extensor digitorum longus). J Anat 168: 73-80, 1990

EISEN A, KARPATI G, CARPENTER S: Reserpine induced alteration of physiological properties and histochemical fiber types in rat skeletal muscle. Exp Neurology 46: 554-565, 1975.

FLIEGEL L, OHNISHI M, CARPENTER MR, KHANNA VK, REITHMEIER RA, MACLENNAN DH: Amino acid sequence of rabbit fast-twitch skeletal muscle calsequestrin deduced from cDNA and peptide sequencing. Proc Natl Acad Sci U S A 84: 1167-71, 1987.

FRANZINI-ARMSTRONG C, KENNEY LJ, VARRIANO-MARSTON E: The structure of calsequestrin in triads of vertebrate skeletal muscle: a deep-etch study. J Cell Biol 105: 49–56, 1987.

GILLESPIE MJ, GORDON T, MURPHY PR: Motor units and histochemistry in rat lateral gastrocnemius and soleus muscles: evidence for dissociation of physiological and histochemical properties after reinnervation. J Neurophysiol 57: 921-937, 1987.

GREEN HJ, KLUG GA, REICHMANN H, SEEDORF U, WIEHRER W, PETTE D: Exercise-induced fibre type transitions with regard to myosin, parvalbumin, and sarcoplasmic reticulum in muscles of the rat. Pflugers Arch 400: 432-438, 1984.

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

HALL-CRAGGS EC, MAX SR, WINES MM, MORELAND TM, HEBEL JR: Central core degeneration after tenotomy in soleus muscles of hyperthyroid rats. Exp Neurol 81: 722-732, 1983.

HÄMÄLÄINEN N, PETTE D: The histochemical profiles of fast fiber types IIB, IID, and IIA in skeletal muscles of mouse, rat, and rabbit. J Histochem Cytochem 41: 733-743, 1993.

HEIZMANN CW, BERCHTOLD MW, ROWLERSON AM: Correlation of parvalbumin concentration with relaxation speed in mammalian muscles. PNAS 79:7243-7247, 1982.

HERBISON GJ, JAWEED MM, DITUNNO JF, SCOTT CM: Effect of overwork during reinnervation of rat muscle. Exp Neurol 41: 1-14, 1973.

HERBISON GJ, JAWEED MM, DITUNNO JF: Recovery of reinnervating rat muscle after cast immobilization. Exp Neurol 85: 239-248, 1984.

HO KW, HEUSNER WW, VAN HUSS J, VAN HUSS WD: Postnatal muscle fibre histochemistry in the rat. J Embryol Exp Morphol 76: 37-49, 1983.

HUDECOVÁ S, VADÁZOVÁ A, SOUKUP T, KRIŽANOVÁ O: Effect of thyroid hormones on the gene expression of calcium transport systems in rat muscle. Life Sci 75: 923-931, 2004.

IANUZZO D, PATEL P, CHEN V, O´BRIEN P, WILLIAMS C: Thyroidal trophic influence on skeletal muscle myosin. Nature 270: 74-76, 1977.

IANUZZO CD, PATEL P, CHEN V, O´BRIEN P: A possible thyroidal trophic influence on fast and slow skeletal muscle myosin. Plasticity of muscle (D.Pette ed.), Walter de Gruyter New York, 1980, pp 593-605.

ITOH M, ITOH K, TAGUCHI S, HIROFUJI C, TAKEUCHI H, ISHIHARA A: Effects of hypobaric hypoxia on fiber type composition of the soleus muscle in the developing rat. Aviat Space Environ Med 63: 583-7, 1992.

JAWEED MM, HERBISON GJ, DITUNNO JF: Denervation and reinnervation of fast and slow muscles. A histochemical study in rats. J Histochem Cytochem 23: 808-827, 1975.

JIANG M, XU A, JONES DL, NARAYANAN N: Coordinate downregulation of CaM kinase II and phospholamban accompanies contractile phenotype transition in the hyperthyroid rabbit soleus. Am J Physiol Cell Physiol 287: C622–C632, 2004.

JORGENSEN AO, SHEN AC, CAMPBELL KP, MACLENNAN DH: Ultrastructural localization of calsequestrin in rat skeletal muscle by immunoferritin labeling of ultrathin frozen sections. J Cell Biol 97: 1573-1581, 1983.

JOUMAA WH, LÉOTY C: A comparative analysis of the effects of exercise training on contractile responses in fast- and slow-twitch rat skeletal muscles. J Comp Physiol [B] 172: 329-338, 2002.

KOVANEN V, SUOMINEN H: Effects of age and life-time physical training on fibre composition of slow and fast skeletal muscle in rats. Pflugers Arch 408: 543-551, 1987.

KRAEMER WJ, STARON RS, GORDON SE, VOLEK JS, KOZIRIS LP, DUNCAN ND, NINDL BC, GÓMEZ AL, MARX JO, FRY AC, MURRAY JD: The effects of 10 days of spaceflight on the shuttle Endeavor on predominantly fast-twitch muscles in the rat. Histochem Cell Biol 114: 349-55, 2000.

KUGELBERG E: Adaptive transformation of rat soleus motor units during growth. J Neurol Sci 27: 269-289, 1976.

LAEMMLI UK: Cleavage of structural proteins during the assembly of the head by bacteriophage T4. Nature 227: 680-685, 1970

LARSSON L, LI X, TERESI A, SALVIATI G: Effects of thyroid hormone on fast- and slow-twitch skeletal muscles in young and old rats. J Physiol 481: 149-61, 1994.

LARSSON L, YU F: Gender-related differences in the regulatory influence of thyroid hormone on the expression of myosin isoforms in young and old rats. Acta Physiol Scand 159: 81-89, 1997.

LEBERER E, SEEDORF U, PETTE D: Neural control of gene expression in skeletal muscle. Calcium-sequestering proteins in developing and chronically stimulated rabbit skeletal muscles. Biochem J 239: 295-300, 1986.

LEWIS DM, LEVI AJ, BROOKSBY P, JONES JV: A faster twitch contraction of soleus in the spontaneously hypertensive rat is partly due to changed fibre type composition. Exp Physiol 79: 377-386, 1994.

LI X, HUGHES SM, SALVIATI G, TERESI A, LARSSON L: Thyroid hormone effects on contractility and myosin composition of soleus muscle and single fibres from young and old rats. J Physiol 494: 555-567, 1996.

LIEBER RL, FRIDÉN JO, HARGENS AR, FERINGA ER: Long-term effects of spinal cord transection on fast and slow rat skeletal muscle. II. Morphometric properties. Exp Neurol 91: 435-448, 1986.

LÍMANOVÁ Z: The thyroid gland-running the show behind the scenes (In Czech). Cas Lek Ces 148: 83-85, 2009.

LUGINBUHL AJ, DUDLEY GA, STARON RS: Fiber type changes in rat skeletal muscle after intense interval training. Histochemistry 81: 55-58, 1984.

MARTIN WD, ROMOND EH: Effects of chronic rotation and hypergravity on muscle fibers of soleus and plantaris muscles of the rat. Exp Neurol 49: 758-771, 1975.

MacLENNAN DH, CAMPBELL KP, REITHMEIER RAF: Calsequestrin. In: CHEUNG WY (ed) Calcium and cell function, vol 4. Academic Press, New York, 151-173, 1983.

MIDRIO M, DANIELI-BETTO D, MEGIGHIAN A, VELUSSI C, CATANI C, CARRARO U: Slow-to-fast transformation of denervated soleus muscle of the rat, in the presence of an antifibrillatory drug. Pflugers Arch 420: 446-450, 1992.

MIYABARA EH, AOKI MS, SOARES AG, SALTAO RM, VILICEV CM, PASSARELLI M, SCANLAN TS, GOUVEIA CH, MORISCOT AS: Thyroid hormone receptor-beta-selective agonist GC-24 spares skeletal muscle type I to II fiber shift.Cell Tissue Res 321: 233-41, 2005.

MRÁČEK T, JEŠINA P, KŘIVÁKOVÁ P, BOLEHOVSKÁ R, ČERVINKOVÁ Z, DRAHOTA Z, HOUŠTĚK J: Time-course of hormone induction of mitochondrial glycerophosphate dehydrogenase biogenesis in rat liver. Biochim Biophys Acta 1726: 217-223, 2005.

MÜLLER S, SEITZ HJ: Cloning of cDNA for the FAD-linked glycerol-3-phosphate dehydrogenase from rat liver and its regulation by thyroid hormones. Proc Natl Acad Sci USA 91: 10581-10585, 1994.

MURPHY RM, LARKINS NT, MOLLICA JP, BEARD NA, LAMB GD: Calsequestrin content and SERCA determine normal and maximal Ca2+ storage levels in sarcoplasmic reticulum of fast- and slow-twitch fibres of rat. J Physiol 587: 443-60, 2009.

MÜNTENER M, VAN HARDEVELD C, EVERTS ME, HEIZMANN CW: Analysis of the Ca2+-binding parvalbumin in rat skeletal muscles of different thyroid states. Exp Neurol 98: 529-41, 1987.

NAKANO H, HAYASHI K, SAITOH A, SAKUMA K, KATSUTA S: Oxidative enzyme activity in the rat soleus muscle and its motoneurons during postnatal maturation. Brain Res Bull 38: 235-238, 1995.

NARUSAWA M: Change in fiber type in partially-denervated soleus muscle of the rat. Tokai J Exp Clin Med 10: 499-507, 1985.

NIEDERLE B, MAYR R: Course of denervation atrophy in type I and type II fibres of rat extensor digitorum longus muscle. Anat Embryol (Berl) 153: 9-21, 1978.

NOVÁK P, ŠKAJEVA M, MARKOVÁ V, SULIMENKO V, SOUKUP T: Expression of calsequestrin in skeletal and heart muscles of rats with altered thyroid status. Physiol Res 57: 24P, 2008.

NOVÁK P, MARKOVÁ V, SULIMENKO V, SOUKUP T: Calcium binding proteins in skeletal and heart muscles of rats with altered thyroid status after heterochronous isotransplantation, Physiol Res 59: 27P, 2010a.

NOVÁK P, ZACHAROVA G, SOUKUP T. Individual, age and sex differences in fiber type composition of slow and fast muscles of adult Lewis strain rats. Comparison with other rat strains. Physiol Res [Epub ahead of print], 2010b.

NOVÁK P, SOUKUP T: Calsequestrin distribution, structure and function, its role in normal and pathological situations and the effect of thyroid hormones. A review. Physiol. Res, submitted.

OISHI Y, YAMAMOTO H, NAGANO M, MIYAMOTO E, FUTATSUKA M: The effects of 2,5-hexanedione and acrylamide on myosin heavy chain isoforms of slow and fast skeletal muscles of the rat. Toxicol Appl Pharm 139: 15-21, 1996.

OKAMURA K, TAUROG A, KRULICH L: Hypothyroidism in severely iodine deficient rats. Endocrinology 109: 464-468, 1981.

OPPENHEIMER JH, SILVA E, SCHWARTZ HL, SURKS ML: Stimulation of hepatic mitochondrial α-glycerophosphate dehydrogenase and malic enzyme by L-triiodothyronine. Characteristics of the response with specific nuclear thyroid hormone binding sites fully saturated. J Clin Invest 59: 517-527, 1977.

OZAKI K, MATSUURA T, NARAMA I: Histochemical and morphometrical analysis of skeletal muscle in spontaneous diabetic WBN/Kob rat. Acta Neuropathol 102: 264-270, 2001.

PADYKULA H, HERMAN E : The specificity of the histochemical method for adenosine triphosphate. J. Histochem. Cytochem. 3: 170-195,1955.

PEDROSA F, SOUKUP T, THORNELL L-E: Expression of an alpha cardiac-like myosin heavy chain in muscle spindle fibres. Histochemistry 95: 105-113, 1990.

PETTE D: Historical Perspectives: plasticity of mammalian skeletal muscle. J Appl Physiol 90: 1119-24, 2001.

PETTE D: The adaptive potential of skeletal muscle fibers. Can J Appl Physiol 27: 423-448, 2002.

PETTE D, STARON RS: Mammalian skeletal muscle fiber type transitions. Int Rev Cytol 170: 143-223, 1997.

PETTE D, STARON RS: Myosin isoforms, muscle fiber types, and transitions. Microsc Res Tech 50: 500-9, 2000a.

PETTE D, STARON RS: Myosin isoforms, muscle fiber types and transitions. Microsc Res Tech 50: 500-509, 2000b.

PETTE D, STARON RS: Transitions of muscle fiber phenotypic profiles. Histochem Cell Biol 115: 359-72, 2001.

POUSSON M, PÉROT C, GOUBEL F: Stiffness changes and fibre type transitions in rat soleus muscle produced by jumping training. Pflugers Arch 419: 127-130, 1991.

PŘENOSIL O, ŽURMANOVÁ J, MALÁČOVÁ D, SOUKUP T: Differentiation of muscle fiber types and expression of myosin heavy chain isoforms in slow and fast rat skeletal muscles – re-evaluated. Phys Res 57: 26-27, 2008.

PULLEN AH: The distribution and relative size of fibre types in the extensor digitorum longus and soleus muscles of the adult rat. J Anat 123: 467-486, 1977.

PUNKT K, PSINIA I, WELT K, BARTH W, ASMUSSEN G: Effects on skeletal muscle fibres of diabetes and Ginkgo biloba extract treatment. Acta Histochem 101: 53-69, 1999.

RAJIKIN MH: Change of fibre type composition in soleus muscle of male and female rats during postnatal development. Mala. App. Bio. 2: 109-113, 1984.

RANVIER L: De quelques faits relatifs à l'histologie et à la physiologie des muscles striés. Arch Physiol Norm Pathol 1: 5, 1873.

RAUCHOVÁ H, ZACHAŘOVÁ G, SOUKUP T*:* Influence of chronically altered thyroid status on the activity of liver mitochondrial glycerol-3-phosphate dehydrogenase in female inbred Lewis rats. Horm Metab Res 36: 286-290, 2004.

RAUCHOVÁ H, MRÁČEK T, NOVÁK P, VOKURKOVÁ M, SOUKUP T: Glycerol-3-phosphate dehydrogenase expression and oxygen consumption in liver mitochondria of female and male rats with chronic alteration of thyroid status. Horm Metab Res 42: 1-5, 2010.

RUEGAMER WR, WESTERFELD WW, RICHERT DA: α-Glycerophosphate dehydrogenase response to thyroxine in thyroidectomized, thiouracil-fed and temperature-adapted rats. Endocrinology 75: 908-916, 1964.

SAKUMA K, YAMAGUCHI A, OHMORI H, KATSUTA S: Nonuniform changes in fibre types in the soleus muscle on the developing rat. Eur J Appl Physiol Occup Physiol 70: 132-137, 1995.

SANDOW A: Excitation-contraction coupling in skeletal muscle. Pharmacol Rev 17: 265–320, 1965.

SCHWALLER B, DICK J, DHOOT G, CARROLL S, VRBOVA G, NICOTERA P, PETTE D, WYSS A, BLUETHMANN H, HUNZIKER W, CELIO MR: Prolonged contraction-relaxation cycle of fast-twitch muscles in parvalbumin knockout mice. Am J Physiol 276: C395-403, 1999

SCHIAFFINO S, REGGIANI C: Molecular diversity of myofibrillar proteins: gene regulation and functional significance. Physiol Rev 76: 371-423, 1996.

SELLINGER OZ, LEE KL: The induction of mitochondrial α-glycerophosphate dehydrogenase by thyroid hormone: Evidence for enzyme synthesis. Biochim Biophys Acta 91: 183-186, 1964.

SCOTT BT, SIMMERMAN HK, COLLINS JH, NADAL-GINARD B, JONES LR: Complete amino acid sequence of canine cardiac calsequestrin deduced by cDNA cloning. J Biol Chem 263: 8958-64, 1988.

SHENOY R, KLEIN I, OJAMAA K: Differential regulation of SR calcium transporters by thyroid hormone in rat atria and ventricles. Am J Physiol Heart Circ Physiol 281: H1690-6, 2001.

SIMARD C, LACAILLE M, VALLIERES J: Effects of hypokinesia/hypodynamia on contractile and histochemical properties of young and old rat soleus muscle. Exp Neurol 97: 106-14, 1987.

SIMONIDES WS, VAN HARDEVELD C: The effect of hypothyroidism on sarcoplasmic reticulum in fast-twitch muscle of the rat. Biochim Biophys Acta 844: 129-41, 1985.

SMERDU V, SOUKUP T: Demonstration of myosin heavy chain isoforms in rat and humans: the specificity of seven available monoclonal antibodies used in immunohistochemical and immunoblotting methods. Eur J Histochem 52: 179-90, 2008.

SOUKUP T, VYDRA J, ČERNÝ M: Changes in ATPase and SDH reactions of the rat extrafusal and intrafusal muscle fibres after preincubations at different pH. Histochemistry 60: 71-84, 1979.

SOUKUP T, PEDROSA-DOMELLÖF F, THORNELL LE: Expression of myosin heavy chain isoforms and myogenesis of intrafusal fibres in the rat muscle spindles. Microsc Res Tech 30:390-407, 1995.

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

SOUKUP T, SMERDU V, ZACHAŘOVÁ G: Fiber type composition of unoperated rat soleus and extensor digitorum longus muscles after unilateral isotransplantation of a foreign muscle in long-term experiments. Physiol Res 58: 253-262, 2009.

STARON RS, KRAEMER WJ, HIKIDA RS, REED DW, MURRAY JD, CAMPOS GE, GORDON SE : Comparison of soleus muscles from rats exposed to microgravity for 10 versus 14 days. Histochem Cell Biol 110:73-80, 1998.

STARON RS, KRAEMER WJ, HIKIDA RS, FRY AC, MURRAY JD, CAMPOS GE : Fiber type composition of four hindlimb muscles of adult Fisher 344 rats. Histochem Cell Biol 111: 117-123., 1999.

STARON RS, PETTE D: The continuum of pure and hybrid myosin heavy chain-based fibre types in rat skeletal muscle. Histochemistry 100: 149-53, 1993.

STEPHENSON GMM: Diversity and plasticity of vertebrate skeletal muscle: insights from hybrid fibres. Braz J morphol Sci 23: 187-194, 2006.

TALMADGE RJ, ROY RR, EDGERTON VR: Persistence of hybrid fibers in rat soleus after spinal cord transection. Anat Rec 255: 188-201, 1999.

THOMAS PE, RANATUNGA KW: Factors affecting muscle fiber transformation in cross-reinnervated muscle. Muscle and Nerve 16: 193-199, 1993.

TIAN LM, FENG DP: The interaction of thyroidectomy with spinal cord transection or cross-inervation in their effects on muscle phenotypic characteristics. Chinese J Physiol Sc 6: 1-12, 1990.

UEDA K, TANIZAWA Y, ISHIHARA H, KIZUKI N, OHTA Y, MATSUTANI A, OKA Y: Overexpression of mitochondrial FAD-linked glycerol-3-phosphate dehydrogenase does not correct glucose-stimulated insulin secretion from diabetic GK rat pancreatic islets. Diabetologia 41: 649-653, 1998.

TRAASETH NJ, HA KN, VERARDI R, SHI L, BUFFY JJ, MASTERSON LR, VEGLIA G: Structural and dynamic basis of phospholamban and sarcolipin inhibition of Ca(2+)-ATPase. Biochemistry. 47: 3-13, 2008.

VADÁSZOVÁ A, ZACHAŘOVÁ G, MACHÁČOVÁ K, JIRMANOVÁ I, SOUKUP T: Influence of thyroid status on the differentiation of slow and fast muscle phenotypes. Physiol Res 53 Suppl 1: S57-61, 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, SOUKUP T: Dual role of thyroid hormones in rat soleus muscle MyHC isoform expression. Physiol Res 56: 833-836, 2007.

VESELY MJ, SANDERS R, GREEN CJ, MOTTERLINI R: Fibre type specificity of haem oxygenase-1 induction in rat skeletal muscle. FEBS Lett 458: 257-260, 1999.

WIGSTON DJ, ENGLISH AW: Fiber-type proportions in mammalian soleus muscle during postnatal development. J Neurobiol 23: 61-70, 1992.

YAMAGUCHI A, SAKUMA K, MORITA I, SOYA H, TAKEDA H, KATSUTA S: Changes in fibre types in rat soleus and plantaris muscles following hypophysectomy and compensatory overload. Acta Physiol Scand 158: 89-95, 1996.

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.

ŽURMANOVÁ J, MALÁČOVÁ D, PŮTA F, NOVÁK P, ŘÍČNÝ J, SOUKUP T: Mass spectrometry analyses of rat 2b myosin heavy chain isoform. Physiol Res 56: 659-662, 2007.

ŽURMANOVÁ J, PŮTA F, STOPKOVÁ R, ŘÍČNÝ J, SOUKUP T: The presence of 2b and 2x/d myosin heavy chain mRNAs in the rat slow soleus muscle confirmed by real time RT-PCR with a newly designed set of primers. Physiol Res 57: 81-82, 2008a.

ŽURMANOVÁ J, PŮTA F, STOPKOVÁ R, SOUKUP T: Real time RT-PCR with newly designed primers confirmed the presence of 2b and 2x/d myosin heavy chain mRNAs in the rat slow soleus muscle. Physiol Res 57: 973-978, 2008b.

10 PUBLICATIONS

NOVÁK P, ŠKAJEVA M, MARKOVÁ V, SULIMENKO V, SOUKUP T: Expression of calsequestrin in skeletal and heart muscles of rats with altered thyroid status. Physiol Res 57: 24P, 2008.

NOVÁK P, ŠKAJEVA M, MARKOVÁ V, SULIMENKO V, SOUKUP T: Changes in calsequestrin expression in hypothyroid rat fast and slow skeletal and cardiac muscles. Physiol Res.57: 81P-82P, 2008.

NOVÁK P, ŠKAJEVA M, MARKOVÁ V, SULIMENKO V, SOUKUP T: Expression of calsequestrin in skeletal and cardiac muscles of hypothyroid rats. 26th Alternative Muscle Club Meeting, University of Leeds, 25P, 2008.

NOVÁK P, MARKOVÁ V, SULIMENKO V, SOUKUP T: Expression of calcium binding proteins in skeletal and heart muscles of rats with altered thyroid status. 36th IUPS International Congress of Physiological Sciences, Kyoto, Japan, 2009.

NOVÁK P, ŘÍČNÝ J, SOUKUP T: Calcium binding proteins in skeletal and heart muscles of rats with altered thyroid status after heterochronous isotransplatation. 5th Congress of MYORES, Malta, 2009.

NOVÁK P, MARKOVÁ V, SULIMENKO V, SOUKUP T: Calcium binding proteins in skeletal and heart muscles of rats with altered thyroid status after heterochronous isotransplantation, Physiol Res 59: 27P, 2010.

NOVÁK P, ZACHAROVA G, SOUKUP T. Individual, age and sex differences in fiber type composition of slow and fast muscles of adult Lewis strain rats. Comparison with other rat strains. Physiol Res [Epub ahead of print], 2010.

RAUCHOVÁ H, MRÁČEK T, NOVÁK P, VOKURKOVÁ M, SOUKUP T: Glycerol-3-phosphate dehydrogenase expression and oxygen consumption in liver mitochondria of female and male rats with chronic alteration of thyroid status. Horm Metab Res 42: 1-5, 2010.

NOVÁK P, SOUKUP T: Calsequestrin distribution, structure and function, its role in normal and pathological situations and the effect of thyroid hormones. A review. Physiol. Res, submitted.

11 SUPPLEMENT 1

FIBRE 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±3.8**	**4.7±3.8**
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 fibres (with no type 1 fibres)

Table 1. A comparison of our data on female and male Lewis inbred strain rats with literature data on the fibre type composition of the soleus muscle of other rat strains. The fibre 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) fibres (mean ± SD or SEM, n. d. = sex not determined).

12 SUPPLEMENT 2

FIBRE 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**

Table 2. A comparison of our data on female and male Lewis inbred strain rats with literature data on the fibre type composition of the EDL muscle of other rat strains. The fibre type composition was determined on the basis of mATPase activity and is expressed as percentages of type 1, type 2A and 2B fibres (mean ± SD or SEM, n. d.=sex not

determined).

Title in Czech

DETERMINACE A REGULACE EXPRESE TĚŽKÝCH ŘETĚZCŮ MYOZINU A PROTEINŮ VÁŽÍCÍCH VÁPNÍK V POMALÝCH A RYCHLÝCH SVALECH POTKANA

Key words

muscle fibre, myosin, mATPase, calsequestrin, parvalbumin, phospholamban, thyroid hormone, GPDH, soleus, extensor digitorum longus