Relations between the Changes in the Turnover Rate of Contractile Proteins, Activation of Satellite Cells and Ultra-Structural Response of Neuromuscular Junctions in the Fast-oxidative-glycolytic Muscle Fibres in Endurance Trained Rats

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Abstract

In fast-oxidative-glycolytic muscle fibers of endurance-trained rats 24 hrs after the last exercise, the RNA:DNA ratio increased significantly, the myosin heavy chain turned over 32% and actin 21% faster than in the control rats. The muscle fibres had about 2.8-fold more satellite cells compared with the controls and a non-uniform structure of axonal terminals. Some of synaptic contact areas contained autophagosomes, extensive membrane structures, coated vesicles, part of terminals was completely destroyed and exposed junctional folds occurred. However, the focal denervation of muscle fibers in most animals was reversible and accompanied by regeneration of new small axonal terminals, ingrowing into pre-existing synaptic grooves. In some of the endplates several small terminals were located within one primary synaptic cleft. It is proposed that biochemical and structural changes in skeletal muscle fibers and neuromuscular junctions of the endurance-trained rats reflects a functionally-determined adaptive process.

Key words: fast-oxidative-glycolytic muscle fibers, endurance-exercise, turnover muscle contractile proteins, satellite cells, axon terminals.

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Skeletal muscle fibres exist as dynamic structures and are capable of adapting to altered functional demands. The structural heterogeneity of muscle tissue reflects its high degree of functional specialization and it is the basis of its plasticity [16]. The fast-oxidative-glycolytic muscle fibres are rather unique in this respect and, at least partially, this is caused by the wide range of recruitment of these fibers during physical activity [20].

Most of the muscle protein synthesis is suppressed and protein breakdown is increased during endurance exercise [4, 9].

Some time after exercise, the synthesis rate for contractile proteins is increased [27] but the turnover rate of certain myofibrillar proteins is varied [22] and depends on the type of skeletal muscle fibres [21]. So, during endurance exercise the myofibrillar protein synthesis rate decreased but 24 hrs after exercise increased and the synthesis rate of sarcoplasmic protein in both cases increased [23]. Some destructive signs in fast-oxidative-glycolytic muscle fibers and motor endplates were observed in endurance-trained rats [24].

The ultrastructural changes in neuromuscular junctions seem to be of special interest because they are essential with respect to the transfer of both motor phasic impulses and neurotrophic signals [29].

It has been shown that exercise caused skeletal muscle hypertrophy as well as hyperplasia might be induced by the activation of satellite cells [2]. After prolonged treadmill running satellite cells were activated not only in muscle fibers exhibiting overt necrosis, but also in intact fibres [8], including fast-oxidative-glycolytic fibres [26].

The aim of this study was to determine the relations between the changes in the turnover rate of contractile proteins, the activation of satellite cells and the ultrastructural response of neuromuscular junctions in fast-oxidative-glycolytic fibres of endurance-trained rats.

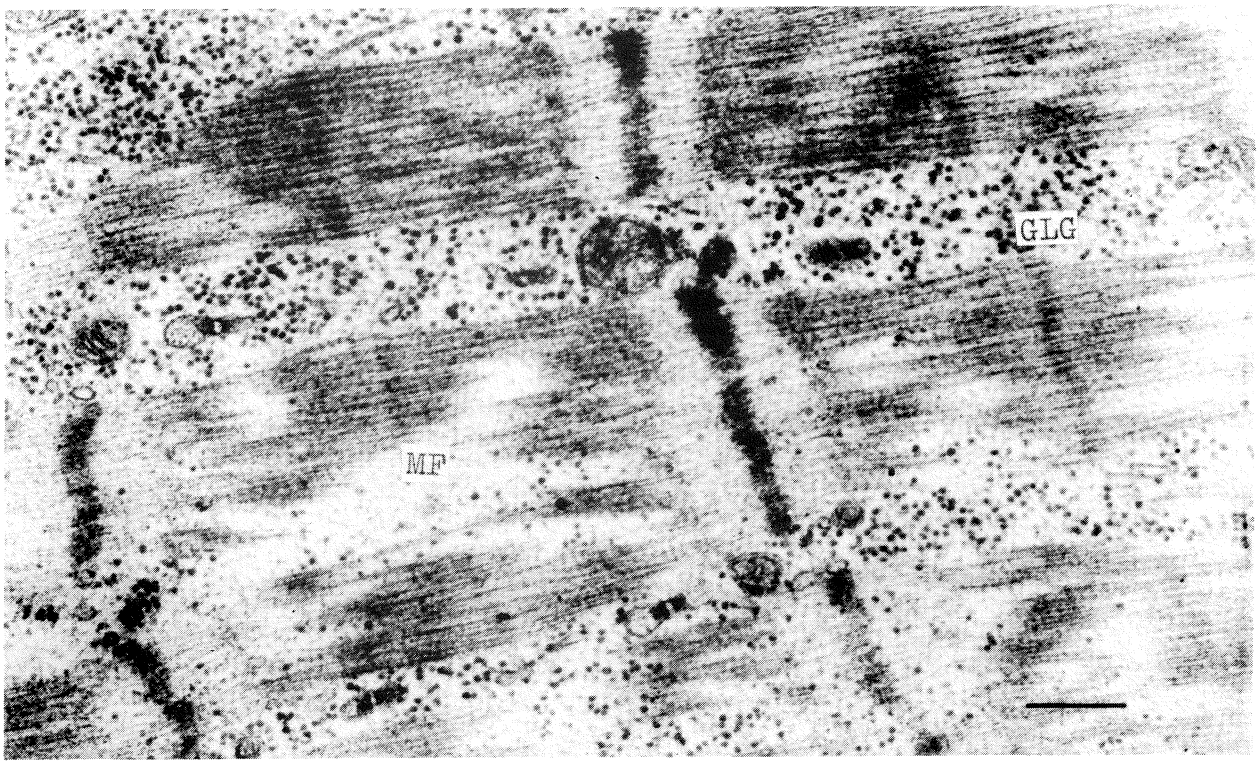

Figure 1. Destruction of myofibrils (MF) in oxidative-glycolytic muscle fibre. GLG - glycogen-like granules, bar corresponds to 1μm.

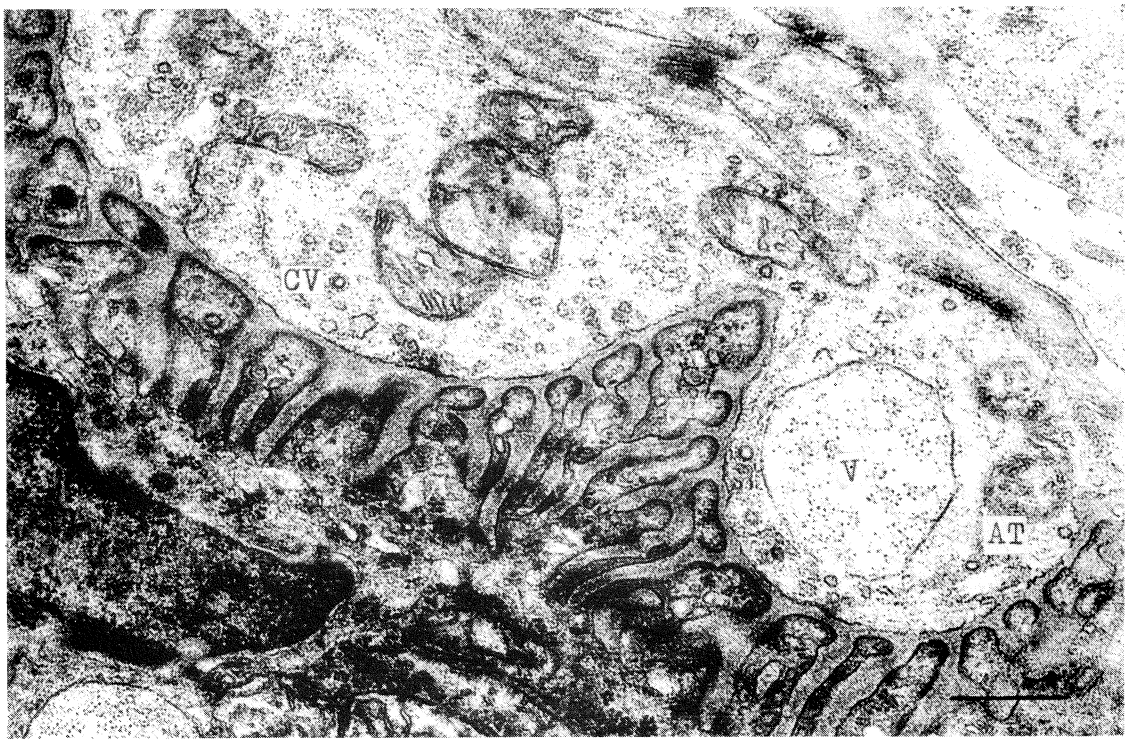

Figure 2. Formation of vacuole-like structures (V) in axonal terminals (AT). CV - coated vesicles, bar corresponds to 0.5 μm.

Materials and Methods

Male rats of the Wistar strain, 16-17 weeks old, were maintained on a diet containing 12% proteins, 28% carbohydrates and 9% lipids. Food and water were given ad libitum. The rats were housed four per cage in plastic cages at 12/12 hrs light/dark period. The rats were assigned to control and trained. The endurance-trained animals were running at 35 m/min 5 days a week for 6 weeks. The duration of the runs was progressively increased (from 5 min to 15 min in the first week, power - 1.3 W and total work per week - 4,300 J) so that in the 6th week of training the animals were running for 60 min per day (power - 1.6 W and total work per week - 27,956 J).

For studies of the fast-oxidative-glycolytic fibers, the muscle quadriceps femoris was dissected out, liberated from fat and connective tissue and was separated into a superficial white region and a deep red portion, which consisted predominantly of fast-oxidative-glycolytic fibres. The fast-oxidative-glycolytic fibers contained cytochrome aa_3 30-35 nmol per g muscle and myoglobin 3.1-3.6 mg per g muscle.

The muscle samples were homogenized in a buffer containing: 50 mM KCl; 10 mM K_2HPO_4; 1 mM EGTA; 1 mM $MgCl_2$; 1 mM 2-dithiothreitol; at pH 7.0. The homogenates were centrifuged at 1,000 g for 10 min, and the supernatant was taken as a sarcoplasmic fraction. The crude myofibrillar pellet was rehomogenized in the same buffer with 0.1% Triton X-100 and centrifuged at 1,000 g for 10 min. The myofibrils were rehomogenized in 0.6 M KCl, 8 mM EGTA, 20 mM $NaHCO_3$, 2 mM ATP, 1 mM 2-dithiothreitol, 50 mM Tris at pH 7.0. The homogenate, which contained myofibrils from 1 g of muscle per 10 ml was kept at 0°C for 20 hrs to extract actomyosin. After that it was centrifuged at 18,000 g for 60 min. The supernatant was diluted 10-fold in 1.75 mM Tris, 0.1 mM 2-dithiothreitol at pH 7.0 to precipitate actomyosin. The protein was then dissolved in 0.6 M KCl, 0.1 mM 2-dithiothreitol and centrifuged at 18,000 g for 30 min. Actomyosin was precipitated and washed twice.

In order to investigate the incorporation of aminoacids into sarcoplasmic proteins and actomyosin L[4.5-^{3}H] Leucine (170 Ci/mmol) was infused intraperitoneally 100 µCi per 100 g body weight. Gel filtration of Sephacryl - S300 was provided as described by Schreurs et al. [19]. SDS electrophoresis was used for the purification of myosin heavy chain (HC) and actin. In order to investigate the turnover rate of contractile proteins in endurance-trained rats, the double isotope method of Funabiki [10] was used. L-[U-^{14}C] Lysine (336 mCi/mmol) 10 µCi per day was discontinued after 5 days and L-[4.5-^{3}H] Lysine (40 Ci/mmol) 100 µCi per day was continued for 12 days. The relative turnover rate of the protein fraction was estimated from the ^{3}H/^{14}C ratios. For the same protein turnover rates, the ^{3}H/^{14}C ratios were expected to be the same. Protein with a higher turnover rate would have a greater ^{3}H/^{14}C ratio. SDS polyacrylamide gel electrophoresis was carried out in 10% acrylamyde gels. The identified fractions were sliced and dissolved in hydrogen peroxide at 50°C overnight and radioactivity was determined with a 1211 Minibeta liquid scintillation counter. Myoglobin was measured by a modification of Reunaforje [17] and cytochrome aa_3 by the method of Schollmeyer [18]. The DNA unit size and RNA:DNA ratio were determined as described by Millward and Waterlow [15].

Muscle samples for ultrastructural studies were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in graded alcohol and embedded in Epon-812. Ultrathin sections were cut from longitudinaly and transversely oriented blocks, stained with uranyl acetate and lead hydroxide, using 3-5 blocks from each animal. The number of satellite cells, containing a nucleus, per 1000 myonuclei in experimental and control groups was calculated by electron microscopy. The satellite cell frequency was determined as a ratio of the nucleus-containing satellite cells divided by the total number of myonuclei including satellite cells' nuclei [8, 28].

In rats of the control (5 animals) and of the experimental (5 rats) groups an electron microscopic examinantion of 114 axonal terminals located in 40 muscle fibers was studied. The data were analyzed by Student's t-test.

Results

In the fast-oxidative-glycolytic muscle fibres the content of cytochrome aa_3 during 6 wk endurance exercise increased from 117.8 ± 20.5 to 166.03 ± 23.0 nmole per mg DNA. Incorporation of labelled leucine into actomyosin fraction immediately after exercise decreased, but 24 hrs after it increased 21% ($p < 0.05$). Incorporation of ^{3}H leucine increased into sarcoplasmic fraction immediately after exercise and stayed high for 24 hrs. Myosin heavy chain synthesis increased 22.2% ($p < 0.05$) and actin synthesis 25% ($p < 0.05$) 24 hrs after last exercise.

In endurance-trained rats 24 hrs after the last exercise, in fast-oxidative-glycolytic muscle fibres, the DNA unit size did not change significantly but RNA:DNA ratio increased from 3.77 ± 0.30 to 4.85 ± 0.33 ($p < 0.01$). At the same time myosin HC turned over 32% ($p < 0.01$) and actin 21% ($p < 0.01$) faster than in control animals.

Destruction of thin and thick myofilaments in trained rats was mostly located in the peripheral myofibrils (Fig.1). Penetration of macrophages under the plasmalemma of some myofibers was observed rather frequently. In the sarcoplasm of muscle fibers there occurred secondary lysosomes, autophagic vacuoles, containing degenerating mitochondria and membranous remnants. In some axon terminals there were lysosome-like bodies, autophagosomes and extensive membrane structures (Fig. 2). Certain axonal terminals were destroyed and in such pre-existing synaptic contact areas only exposed junctional folds could be seen.

Simultaneously signs of intracellular regeneration processes could be registered in the muscle fibers of the trained rats. In clusters of mitochondria, situated under the plasmalemma, among large forms were also small ones

Figure 3. Two satellite cells (SC) in one muscle fibre (MF). Bar corresponds to 1 μm.

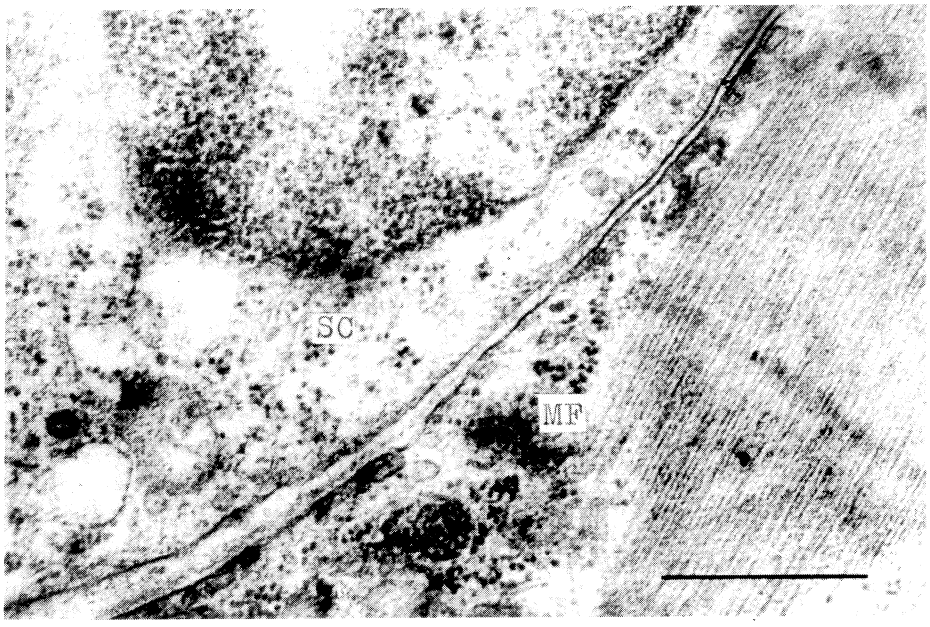

Figure 4. Penetration of basal laminia between muscle fibre (MF) and satellite cell (SC). Bar corresponds to 0.5 μm.

with occasional cristae. In peripheral sarcoplasm there occurred many free ribosomes, polysomes and rough endoplasmic reticulum. Under the basal lamina of muscle fibers the satellite cells were located in extra- and perisynaptic areas. These were at distinct stages of differentiation. Many of them had increased nucleus-cytoplasmic ratios and numerous organelles. In the trained rats extra- and perisynaptic satellite cells formed groups of two or more cells (Fig. 4) which was not the case in the investigated control animals. Sometimes basal lamina penetrated between the muscle fibre and the satellite cell (Fig. 5). The satellite cell frequency in fast-oxidative- glycolytic muscle fibers in endurance-trained rats increased by 2.8-fold compared with the controls ($p < 0.001$). It seems that in trained rats perisynaptic satellite cells were more evident than in control animals.

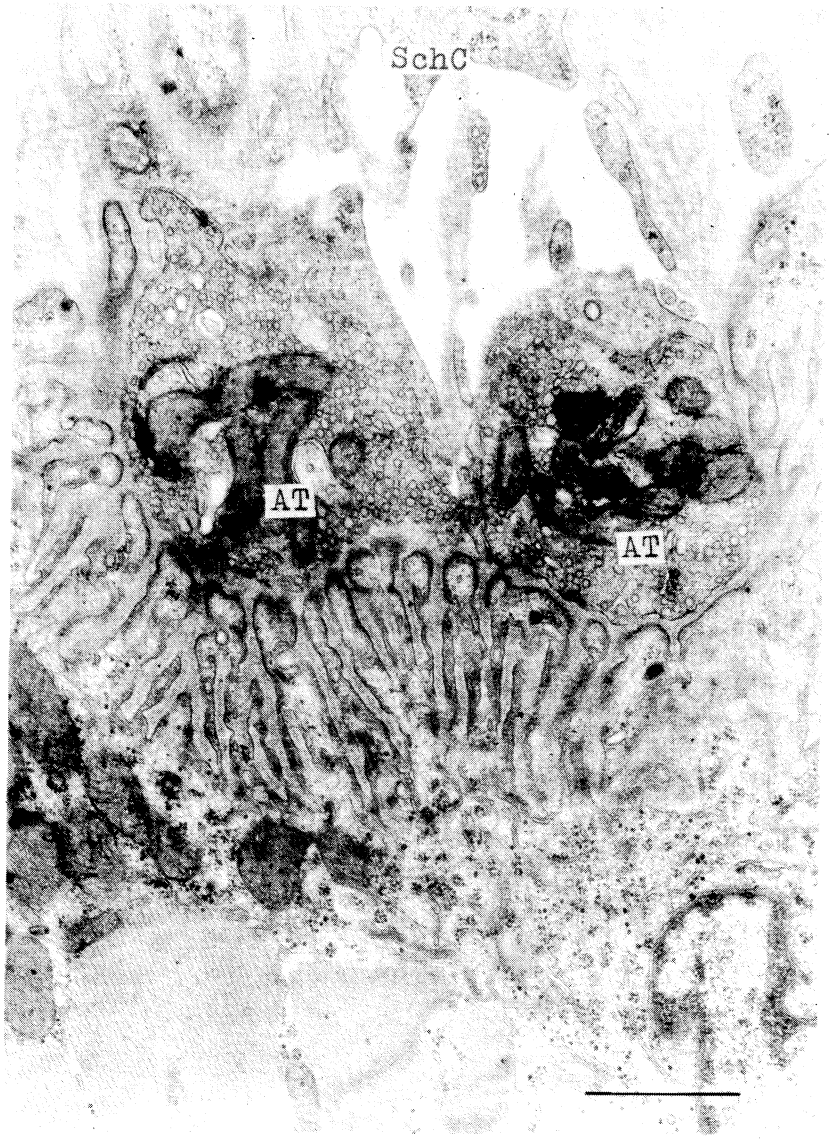

Figure 5. Two regenerated axonal terminals (AT) open in one primary synaptic cleft, bar corresponds to 1 μm.

Fast-oxidative-glycolytic muscle fibres had elongated or small-sized circular axonal terminals. In the same animal some of the axonal terminals were rich in smooth electron-lucent vesicles while other terminals had coated vesicles and were abundant in mitochondria. The signs of focal denervation were one of the ultrastructural features of trained rats myofibers. The newly-formed terminals were ingrown into pre-existing synaptic grooves and were smaller in size than the destroyed ones at the site where they appeared. Sometimes, in trained rats ingrowing small terminals had not yet established contact with old (pre-existing) junctional folds. Not seldom two axonal terminals were located in one primary synaptic cleft and they were isolated from each other by Schwann cell cytoplasm (Fig. 5). There was sometimes only an axonal terminal separated by collagen which penetrates into primary synaptic cleft and exposed junctional folds were formed (Fig. 6). The numerous post-synaptic sarcoplasmic organelles, associated with protein synthesis structures such as ribosomes, polysomes, rough endoplasmic reticulum, a few Golgi complexes, junctional myonuclei forming invaginations containing myofilaments and other structures were observed in endurance-trained rats. In this region there was a great number of mitochondria, part of them dividing along cristae.

Discussion

It was shown that the turnover rate of muscle contractile proteins gives us valuable information about the physiological conditions of the contractile apparatus during en-

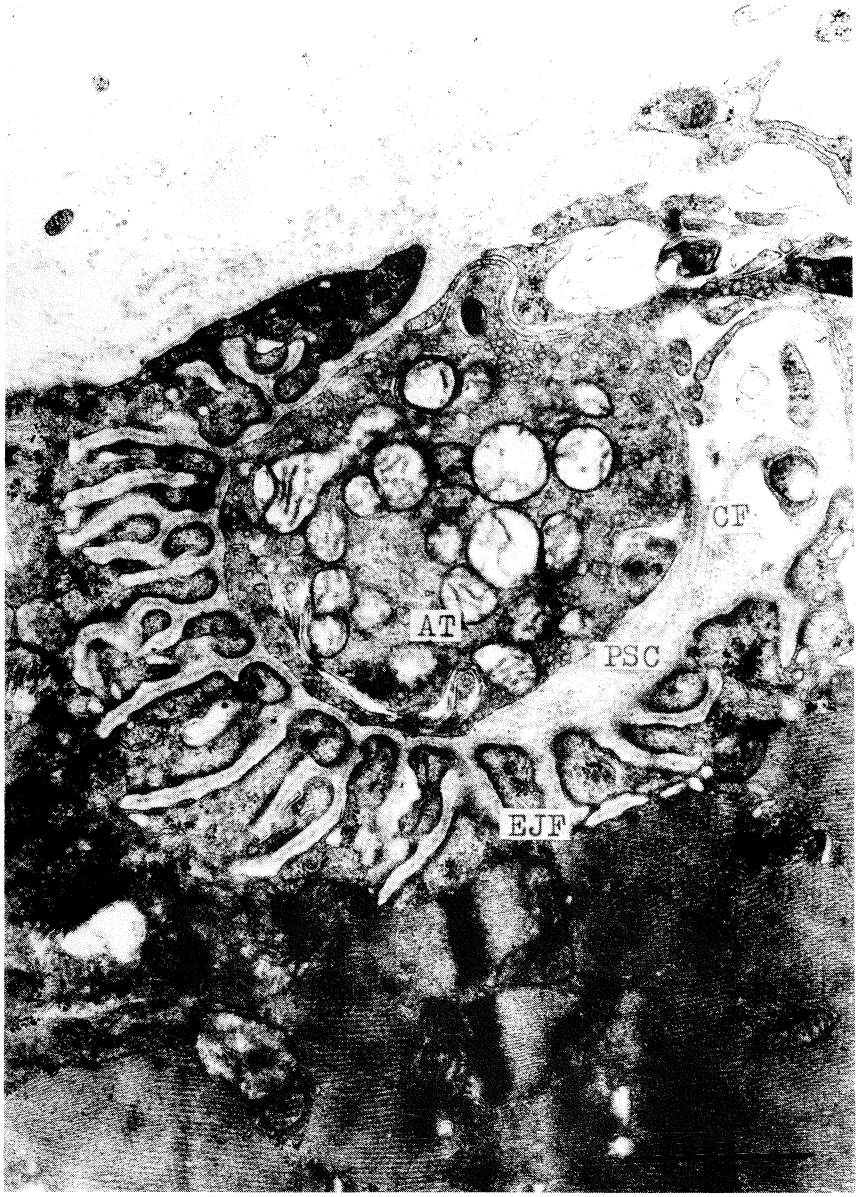

Figure 6. Penetration of collagen fibrils (CF) into primary synaptic cleft (PSC) separating axonal terminal (AT) from junctional folds (JF). Some exposed junctional folds (EJF) can be seen, bar corresponds to 1 μm.

durance exercise as well as during the recovery period after exercise [21]. Two main muscle contractile proteins myosin and actin have similar turnover rates during exercise and in the recovery period after long-lasting exercise [22]. During the endurance-type exercise, myosin HC and actin had a slower turnover rate than in the pre-existing period. At the same time myosin LC had a significantly faster turnover rate than that observed in control rats [22].

As endurance training causes an increase in the number of satellite cells as well as intensification of protein synthesis in the fast-oxidative-glycolytic muscle fibres largely on account of biosynthesis of sarcoplasmic proteins, there are grounds to believe that satellite cells in trained rats replenish the fraction of sarcoplasmic proteins when the proteins are separated into sarcoplasmic and myofibrillar fractions. Thus the elevated incorporation of labelled leucine into sarcoplasmic fraction of fast-oxidative-glycolytic fibers may be caused by satellite cells in which the involvement of aminoacid is possibly more intensive than in muscle fibers [23].

The other biochemical changes in myofibers were also correlated with their ultrastructure, including de- and regenerating signs. Increased RNA:DNA ratio in muscle fibers of endurance-trained rats was confirmed by the existence of numerous ribosomes, polysomes and rough endoplasmic reticulum in peripheral sarcoplasm, in postsynaptic areas, and in satellite cells. It was shown that the

forming small new muscle fibers also contained many polysomes and other organelles [26].

One of the factors for the activation of satellite cells may be denervation of muscle fibers, as we observed in trained rats [26]. The existence of some destroyed neuromuscular junctions and exposed junctional folds are evidence of focal denervation of myofibers that had not been recorded in the investigated control rats. Hanzlikova and co-workers [11] showed that when compensatory hypertrophy of the rat soleus was combined with its denervation, the number of satellite cells increased 5-fold compared with the normal muscle.

Partial degeneration of some axonal terminals caused also the decrease in the whole area of synaptic contact in the neuromuscular junction [6]. The focal denervation of some muscle fibres during endurance exercise was reversible in most cases and was accompanied by the regeneration of axonal terminals. The process of renewal of the axonal terminals during endurance-exercise is similar to the regeneration of terminals after the instrumental injury of the nerve [12, 13] and to the aging reorganization of rat soleus neuromuscular junctions [6, 7]. In normal animals there was an age-dependant reorganization of neuromuscular junctions with the formation of exposed junctional folds [6, 7]. This process was connected with the increasing load to the muscle apparatus due to growth in the body mass of the animals [3, 25]. In rats it occurs at an age of 37-43 weeks [7]. However, since in the investigated neuromuscular junctions of the control rats no substantial changes of pre- and postsynaptic structures were revealed we believed that the process or reorganization of neuromuscular junctions in trained rats can be explained by the effect of increased muscular activity.

In endurance-trained rats the focal denervation of muscle fibers takes place possibly at the level of axon terminals, that is why the repair of new terminals was completed by the end of the training in most cases. The different structure of the axonal terminals in the same experimental rat was indicative of the injury of the terminals in different periods of the training cycle. With the damage of the axon terminals at a later period the process of reinnervation of the neuromuscular junctions was not entirely completed by the end of the training and axonal terminals were continuing to ingrow into the pre-existing synaptic grooves [2]. The new neuromuscular contacts were also characterized by the presence of two terminals at the same primary synaptic cleft [6]. The formation of new axon terminals could be based on two mechanisms - either as a result of the activity of motoneuron growth factors which were directed to the injured terminal, or the stimulus to the axon growth was inside the muscle fiber where the neuroactive agents promoting the growth of the terminals could be synthesized [1, 5]. The action of the latter mechanism was supported by the experiments with adaptation of the diaphragm muscle of the mice trained to the conditions of hypobaric hypoxia [1]. In this case in the sarcoplasmic reticulum acetylcholinesterase was accumulated which might serve as a stimulus to the formation of new motor end-plates [1]. Thus, increased muscular activity led to a considerable reorganization of neuromuscular junctions in about six-month-old rats.

Hence, changes in the turnover rate of muscle contractile proteins in fast-oxidative-glycolytic muscle fibers in endurance-trained rats clearly reflect the physiological conditions of the contractile apparatus, have good correlations with the activation of satellite cells and reorganization of neuromuscular junctions and can be possibly regarded as a manifestation of the functionally-determined adaptive process caused by an increase in the muscular activity of the animals.

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References

[1] Appell H-J: Proliferation of motor end-plates induced by increased muscular activity. *Int J Sports Med* 1984; 5: 125-129.

[2] Appell H-J, Forsberg S, Hollmann W: Satellite cell activation in human skeletal muscle after training: evidence for muscle neoformation. *Int J Sports Med* 1988; 9: 297-299.

[3] Barker D, Ip MC: Sprouting and degeneration of mammalian motor axons in normal and de-afferentiated skeletal muscles. *Proc Roy Soc (London)*, ser. B 1966; 163: 538-554.

[4] Booth F, Watson P: Control of adaptations in protein levels in response to exercise. *Fed Proc* 1985; 44: 2293-2300.

[5] Brown MC, Holland RL, Ironton R: Nodal and terminal sprouting from motor nerves in fast and slow muscles of the mouse. *J Physiol (London)* 1980; 306: 493-510.

[6] Cardasis CA: Ultrastructural evidence of continued reorganization at the aging (11-26 months) rat soleus neuromuscular junction. *Anat Rec 1983; 207: 399-415.*

[7] Cardasis CA, Padykula HA: Ultrastructural evidence indicating reorganization at the neuromuscular junction in the normal rat soleus muscle. *Anat Rec* 1981; 200: 41-59.

[8] Darr KC, Schultz E: Exercise-induced satellite cell activation in growing and mature skeletal muscle. *J Appl Physiol* 1987; 63: 1816-1821.

[9] Dohm G, Kasperek G, Tapscott E, Buscher G: Effect of exercise on synthesis and degradation of muscle protein. *Biochem J* 1980; 188: 255-262.

[10] Funabiki R, Gassens R: Heterogeneous turnover of myofibrillar protein. *Nature (London)* 1972; 216: 1219-1220.

[11] Hanzlikova V, Mackova EV, Hnik P: Satellite cells of the rat soleus muscle in the process of compensatory hypertrophy combined with denervation. *Cell Tiss Res* 1975; 160: 411-421.

[12] Manolov SA: Ultrastructural studies on regeneration of neuromuscular synapses of rat plantaris muscle. *Compts rendus de l'Academie bulgare des Sci* 1975; 28: 853-855.

[13] Miledi R, Slater CR: Electrophysiology and electronmicroscopy of rat neuromuscular junctions after nerve degeneration. *Proc Roy Soc B* 1968; 169: 289-306.

[14] Millward D, Waterlow J: Effect of the nutrition on protein turnover in skeletal muscle. *Fed Proc* 1978; 37: 2283-2290.

[15] Moss F, Leblond C: Satellite cell as the source of nuclei in muscles of growing cats. *Anat Rec* 1971; 170: 421-436.

[16] Pette D, Staron RS: Cellular and molecular diversities of mammalian skeletal muscle fibres. *Rev Physiol Pharmacol* 1990; 116: 1-76.

[17] Reis D, Wooten G: The relationship of the blood flows to myoglobin, capillary density, and twitch characteristics in red and white skeletal muscle in cat. *J Physiol (London)* 1970; 210: 121-135.

[18] Schollmeyer P, Klingenberg M: Über den cytochrom inhalt tierischer Gewebe. *Biochem Z* 1962; 335: 426-439.

[19] Schreurs V, Boekholt H, Koopmanschap R, van Rotterdam W: Relative synthesis rate of individual muscle proteins: a new approach. *Neth J Arg Sci* 1985; 33: 297-302.

[20] Seene T: Turnover rate of contractile proteins in exercised skeletal muscle (In Russian). *Adv modern Biol* 1990; 110: 290-305.

[21] Seene T, Alev K: Effect of muscular activity on the turnover rate of actin and myosin heavy and light chains in different types of skeletal muscle *Int J Sports Med* 1991; 12: 204-207.

[22] Seene T, Alev K, Pehme A: Effect of muscular activity on the turnover rate of actin, myosin heavy and light chains in different types of skeletal muscle. I Changes in the turnover rate of myosin and actin during and after single-bout physical activity. *Int J Sports Med* 1986; 7: 287-290.

[23] Seene T, Umnova M, Alev K, Pehme A: Adaptation of oxidative-glycolytic muscle cells to endurance training (In Russian). *Acta et Commentationes Universitatis Tartuensis* (Edited by T. Seene). Tartu 1987; 773: 144-156.

[24] Studitsky AN, Seene TP, Umnova MM: Ultrastructural changes of axomuscular synapses to increased locomotor activity. (In Russian). *Dokl Akad Nauk USSR* 1985; 281: 426-428.

[25] Taffery AR: Growth and degeneration of motor end-plates in normal cat hind limb muscles. *J. Anat* 1971; 110: 221-247.

[26] Umnova MM, Seene TP: The effect of increased functional load on the activation of satellite cells in the skeletal muscle of adult rats. *Int J Sports Med* 1991; 12: 501-504.

[27] Wenger H, Wilkinson J, Dalloire J, Nihei T: Uptake of ^{3}H-Leucine into different fractions of rat skeletal muscle following acute endurance and sprint exercise. *Eur J Appl Physiol* 1981; 47: 83-92.

[28] Wokke JHJ, Van Den Oord CJM, Leppink GJ, Jennekens FGI: Perisynaptic satellite cells in human external intercostal muscle: a quantitative and qualitative study. *Anat Rec* 1989; 223: 174-180.

[29] Zhenevskaya RP: *Neurotrophic regulation of the plastic activity of muscle tissue* (In Russian). Moscow, Nauka Publ, 1974.