Scand. J. Lab. Anim. Sci. No. 1. 1996. Vol. 23

The effect of activity on the rat skeletal muscle contractile apparatus

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Introduction

Laboratory animals are often used in studying the effect of exercise on structures of the organism. One of the simplest and therefore most frequently used ways of determining the doses of experimental load is swimming. The main shortcoming of swimming is its very low intensity. Attaching of additional loads to the animal's body changes the position of its body in water and thereby also the groups of the working muscles. These circumstances make it very difficult to compare the studies carried out in case of different swimming bouts. Taking into account the information given above, the use of treadmill running makes it possible to determine the speed of animals according to the type of movement with more accuracy. In order to compare the nature of work done by different kinds of animals and humans the intensity of O_2 consumption during work has been registered (*Shepard & Gollnick* 1976).

The purpose of the present study was to establish different models for the rat running exercise and compare the effect of different exercise protocols (total work and power of the exercise) on the turnover rate of myosin heavy chains (myosin HC) and on the myosin HC isoform pattern in different types of skeletal muscle fibers of the rat.

Materials and Methods

Male rats of the Wistar strain (National Laboratory Animal Center, Kuopio, Finland) 16-17 weeks old, were maintained on a constant diet SDS-RM 1 (C) 3/8 (SDS, Witham, England). Food and water were given *ad libitum*. The rats were housed four per cage in plastic cages at 12h:12h light:dark period. For studies of the fast-glycolytic (type IIB) and fast-oxidative-glycolytic (type IIA) fibers, the *m.quadriceps femoris* was dissected and cytochromes aa_3 and myoglobin were used as markers for the fast-glycolytic and fast-oxidative-glycolytic type of muscle fibers (*Seene & Alev* 1991). The *soleus* muscle, which consisted predominantly of slow-oxidative fibers, was used for studies on the slow-twitch fibers (type I) and *m. gastrocnemius* was used for studies as the mixture of these three muscle fiber types (*Seene & Alev* 1991). Myoglobin (*Reis, Wooten* 1970) and cytochrome aa_3 (*Schollmeyer & Klingenberg* 1962) content were used as biochemical markers in separating the fibers. The cytochrome aa_3 content in fast-glycolytic muscle fibers was 7.6-9.8 ìmol/g of wet muscle tissue and the myoglobin content was 0.70-0.96 mg/g of the wet muscle tissue. In fast-oxidative-glycolytic muscle fibers the respective figures were 30.8-33.7 ìmol/g and 3.01-3.20 mg/g. At the ultrastructural level the types of muscle fibers were determined on the basis of their morphological differences (*Seene & Umnova* 1992). Dynamics of the turnover rates of contractile proteins was estimated by single and double isotope method (*Seene & Alev* 1991). The content of glycogen was determined by *Lo et al.* (1970) and the intensity of glyogenolysis was determined as described earlier (*Seene et al.* 1980). The myosin heavy chain (myosin HC) isoforms were separated from *m. Plantaris* using 5-8 % gradient SDS-PAGE (*Bär & Pette* 1988, *Sugiura & Murakami* 1990). The staining reactions were quantified by evaluating densitometrically the area of the respective bands.

Running with the speed of 35 m/min.

In the first week of training the total work was 4 294 ±117 J and in the 6th week 27 950±1 050 J. The total energy expenditure during 6 weeks of exercise training was 25 000 cal. The intensity of exercise during this period increased from 1.30±0.04 to 1.58±0.07 W (Table 1).

Table 1. The characterization of the total work done by animals (A) and the power of exercise (N) by different running protocols during 6 weeks of exercise training.

RUNNING REGIMEN	WEEKS	A (J)	N (W)
35 m/min	I week	4 294 ± 117	1.30 ± 0.04
	VI week	27 950 ± 1 050	1.58 ± 0.07
65 m/min	I week	3 990 ± 140	1.71 ± 0.06
	VI week	10 370 ± 487	2.90 ± 0.13
95 m/min	I week	1 870 ± 60	2.6 ±0.08
	VI week	1 620 ± 81	4.1 ± 0.2

Running with the speed of 65 m/min
In the first week of training the total work was 3 990±140 J and in the 6th week 10 370±487 J. The total energy expenditure during 6 weeks of exercise was 11 390 cal. The intensity of exercise during this period increased from 1.71±0.06 to 2.90±0.13 W (Table 1).

Running with the speed of 95 m/min
In the first week of training the total work was 1 870±60 J and in the 6th week 1 620±81 J. The total energy expenditure during 6 weeks of exercise training was 2 570 cal. The intensity of exercise training during this period increased from 2.60±0.08 to 4.1±0.2 W (Table 1).
The total work (A) done by animals and the power of exercise (N) was calculated by the following formulas:

$$A=m(V/t \times 60 + 9.81) \times S \qquad N=A/t$$

m - body weight (kg); V - running speed (m/min); t - running time (sec); S - running distance (m).
Statistical differences between the main values were considered significant at $p<0.05$ using the Student's test (in case of the turnover rate of the myosin heavy chain) and analysis of variance (ANOVA) (in case of the changes in the myosin heavy chain isoform percentage).

Results
In skeletal muscles during low-intensive exercise the slow oxidative muscle fibers are the first to be recruited. As the exercise intensity increases, also fast oxidative-glycolytic muscle fibers, and in the case of especially vigorous contractions, fast glycolytic muscle fibers become involved. This increase in work intensity is also characterized by the intensity of glycogenolysis in different types of muscle fibers. Thus, in running regimen 35 m/min the intensity of glycogenolysis in slow oxidative and fast oxidative-glycolytic muscle fibers was about four times higher than in the fast glycolytic muscle fibers. With the increase in running speed to 65 m/min the intensity of glycogenolysis in the slow oxidative muscle fibers increased about four times, in the fast oxidative-glycolytic muscle fibers about 40 times, and in the

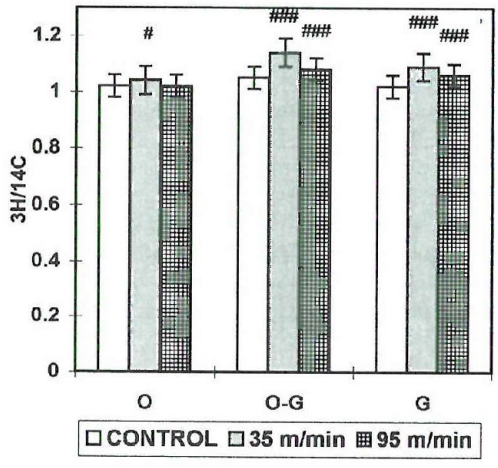

Figure 1. The changes of the turnover rate ($^3H/^{14}C$) of myosin heavy chains after different running regimens. O - oxidative muscle fibres, O-G - oxidative-glycolytic muscle fibers, G - glycolytic muscle fibers. Values are means ± SE, n = 8. ### - $p < 0.01$; # - $p < 0.05$ - significantly different in comparison with control group.

fast glycolytic muscle fibers about 140 times. A further rise in the work intensity increased the intensity of glycogenolysis only in the fast glycolytic muscle fibers.

In 35 m/min running rats, 24 hours after the last exercise session in all types of skeletal muscle fibers, the myosin HC turned over faster than in control animals, in slow oxidative fibers for 22% ($p<0.05$), in fast oxidative-glycolytic fibers for 31% ($p<0.01$) and in fast glycolytic fibers for 17% ($p<0.01$) (Figure 1). In 95 m/min running rats the myosin HC turned over faster only in fast oxidative-glycolytic and fast glycolytic fibers (18% and 25% respectively) (Figure 1). Differences in the turnover rate of myosin HC between 35 m/min and 95 m/min running rats occurred only in slow-oxidative muscle fibers. The turnover rate of myosin light chains (myosin LC) remained at the control animals' level 24 hours after the last exercise (data not shown). In 95 m/min running animals the ratio of myosin HC and actin decreased in glycolytic type of muscle fibers compared with the control group ($p < 0.05$) (Figure 2), in 35 m/min running rats we did not observe significant changes in the ratio of myosin HC and actin. The running with high intensity (95 m/min) induced significant alterations in myosin HC isoform pattern in *m. Plantaris*. After 6 weeks of high-intensive running exercise programme, there was a significant decrease (27,2%, $p < 0.01$) in the percentage of myosin HC I (Figure 3A). At the same time, the percentage of myosin HC IIa/d showed an increase (10.4%, $p < 0.01$) (Fig.3A). The percentage of myosin HC IIb decreased 25.02% ($p < 0.05$) in 95 m/min running rats in *m. Plantaris* (Figure 3A). At the same time the turnover rate of total myosin HC in *m. Plantaris* increased 25.6% ($p <0.05$) (Figure 3B).

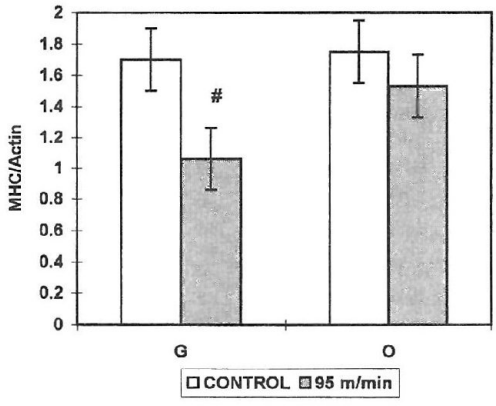

Figure 2. The changes in the ratio of myosin heavy chains and actin after short-duration running with high intensity. G - glycolytic muscle fibers, O - oxidative muscle fibers. Values are means ± SE, n = 8; # - $p < 0.05$ - significantly different in comparison with control group.

Discussion

Exercise influences the whole organism, including the muscular system. Repeated exercise makes the organism adapt itself to it. Adaptive changes in the rat organism and in the skeletal muscles depend on the nature of exercise (*Seene* 1990, *Seene & Alev* 1991, *Seene & Umnova*, 1992). The muscular system is characterized by a dynamic state, reflected in the continous process of protein synthesis and degradation in the muscular tissue (*Booth & Watson* 1985). These processes guarantee the renewal or turnover of muscle proteins. Depending on the activity of skeletal muscles, these two contradictory processes - synthesis and degradation - can be balanced or shifted to one or the other side. If protein synthesis prevails over degradation in the muscle, intensive growth occurs (*Wenger et al.* 1981). In practice a good example is offered by skeletal muscle hypertrophy following the strength type of exercise (*Goldspink* 1983). If intensive degradation of muscle protein prevails over the the intensity of synthesis, muscular atrophy develops. A typical example here is exercise myopathy which occurs as a result of exhaustive exercise, which has damaging effect on muscle fibers and causes their atrophy (*Seene & Viru* 1982, *Salminen & Vihko* 1983, *Komulainen et al.* 1993). Both in muscular hypertrophy and in muscular atrophy the changes that take place are revealed on different levels: molecular, ultrastructural, micro- and macrostructural.

Our data demonstrated that long-term running with relatively low intensity causes more rapid turnover rate in all types of skeletal muscle fibers of rat. The running of short duration with high intensity causes more rapid turnover rate of

myosin heavy chains only in both fast types of skeletal muscle fibers. The running of short duration with high intensity causes the decrease in the ratio of myosin heavy chains and actin and it seems to be conditioned by more intensive synthesis of actin.

The plasticity of skeletal muscle fibers is based on the fact that most myofibrillar proteins exist as sets of isoforms covering ranges of functional properties (*Pette & Staron* 1990). The changes in the myosin HC isoforms induced by different exercise programmes have been investigated in many cases, mostly by endurance type of exercise (*Sugiura et al.* 1992, *Schluter & Fitts* 1994). *Sugiura et al.* (1992) using treadmill running with different duration of the exercise programme showed the increase in the myosin HC IIa and decrease in myosin HC IIb. Under the electrophoretical conditions used we were not able to separate myosin HC IIa from myosin HC IId into separate bands and our data show the increase in the percentage of myosin HC IIa/d after 6 weeks of high intensity running exercise. This makes it difficult to determine in which type of myosin HC isoform the changes take place. Using high-frequency electrical stimulation *Schiaffino et al.* (1988) showed a 80% increase in the myosin HC IIx (which corresponds to myosin HC IId) in *m. Soleus*. This indicates that more frequent activation of muscle fibers may cause the increase of myosin HC IId content in the skeletal muscle. It is surprising that 95 m/min running caused the decrease in the percentage of myosin HC IIb. This fact may be explained with the high intensity and relatively large number of the exercise sessions per day. Similar findings were shown by *Ausoni et al.* (1990) in the *m. Extensor Digitorum Longus* using 2 different models for high-frequency electrical stimulation. At the same time the turnover rate of total myosin HC increased significantly in *m. Plantaris*. More detailed information about myosin HC and skeletal muscle fibers during high intensive exercise may give the turnover rates of different myosin HC isoforms.

Acknowledgements

This study was supported by Estonian Scientific Foundation grant nr. 1065.

Summary

In 35 m/min running rats the myosin heavy chains have a faster turnover rate in all three types of skeletal muscle fibers. In the case of 95 m/min running rats the myosin heavy chains turned over faster only in fast-glycolytic and fast-oxidative-glycolytic type of muscle fibers. The turnover rate of myosin light chains remained at the control animals' level. 95 m/min running exercise caused significant alterations in myosin heavy chain isoforms percentage -the decrease in type I and IIb of

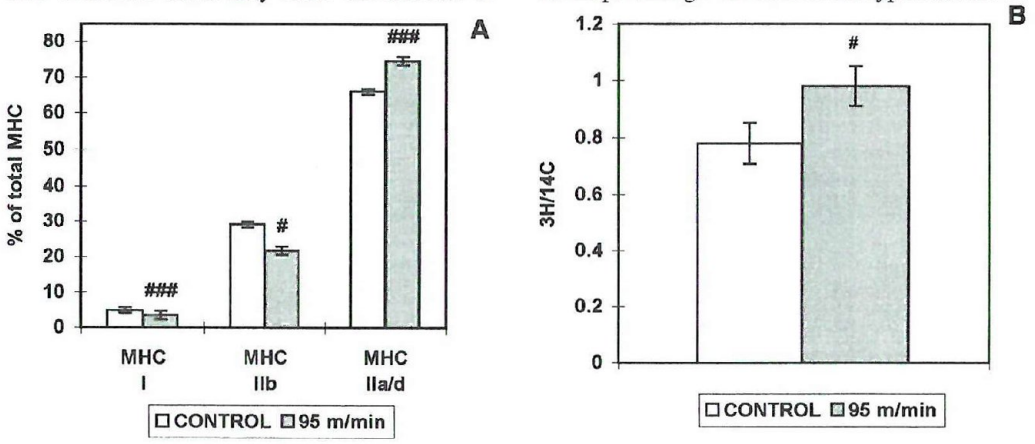

Figure 3. The changes in the myosin heavy chain (MHC) isoforms in the rat *m . Plantaris* after 6 weeks 95 m/min running exercise (A) and the turnover rate of the total MHC in the rat *m. Plantaris* (B). Values are means ± SE, n = 8; # - $p<0.05$; ### - $p<0.01$ - significantly different in comparison with control group.

myosin heavy chain isoforms and the increase in type IIa/d of myosin heavy chain isoforms. At the same time the turnover rate of the total myosin heavy chain fraction showed the increase.The ratio of myosin heavy chains and actin decreased in glycolytic type of muscle fibers in 95 m/min running rats.

References

Ausoni S, L Gorza, S Schiaffino, K Gundersen & T Lomo: Expression of Myosin Heavy Chain Isoforms in Stimulated Fast and Slow Rat Muscles. The Journal of Neuroscience, 1990, *10*, 153-160.

Booth F & P Watson: Control of Adaptations in Protein Levels in Response to Exercise. Fed Proc, 1985, *44*, 2293-2300.

Bär A & D Pette: Three Fast Myosin Heavy Chains in Adult Rat Skeletal Muscle FEBS Lett, 1988, *235*, 153-155.

Goldspink G: Alteration in Myofibril Size and Structure during Growth Exercise and Changes in Enviromental Temperature. In: Handbook of Physiology. 1983, Section 10, 539-554, Bethesda, Maryland.

Komulainen J, R Pitkänen & V Vihko: Muscle Water Content and Exercise-Induced Damage in Mice After Submaximal Running. Med Sci Res, 1993, *21*, 111-113.

Lo S, J Russell & A Taylor: Determination of Glycogen in Small Tissue Samples. J Appl Physiol, 1970, *28*, 234-236.

Pette D & R Staron: Cellular and Molecular Diversities of Mammalian Skeletal Muscle Fibers. Rev Physiol Biochem Pharmacol, 1990, *116*, 1-76.

Reis D & G Wooten: The Relationship of Blood Flows to Myoglobin, Capillary Density, and Twitch Characteristics in Red and White Skeletal Muscle in Cat. J Physiol (Lond), 1970, *210*, 121-135.

Salminen A & V Vihko: Susceptibility of Mouse Skeletal Muscles to Exercise Injuries. Muscle & Nerve, 1983, *6*, 596-601.

Schiaffino S, S Ausoni, L Gorza, L Saggin, K Gundersen & T Lomo: Myosin Heavy Chain Isoforms and Velocity of Shortening of Type 2 Skeletal Muscle Fibres. Acta Physiol Scand, 1988, *134*, 575-576.

Schluter J & R Fitts: Shortening Velocity and ATPase Activity of Rat Skeletal Muscle Fibers: Effects of Endurance Exercise Training. Am J Physiol 1994, *266* (Cell Physiol 35), C1699-C1713.

Schollmeyer P & M Klingenberg: Über den Cytochrom Gehalt tierischer Gewebe. Biochem Z, 1962, *335*, 426-439.

Seene T, R Masso & K Alev: Effects of Increased Functional Activity on Contractile Function of Skeletal Muscles. Sechenov Physiological Journal of the USSR, 1980, LXVI, *3*, 354-361, (in Russian).

Seene T & A Viru: The Catabolic Effect of Glycocorticoidds on Different Types of Skeletal Muscle Fibers and its Dependence upon Muscle Activity and Interaction with Anabolic Steroids. J Steriod Biochem, 1982, *16*, 394-352.

Seene T. Turnover Rate of Contractile Proteins in Exercised Skeletal Muscle. Adv Modern Biol, 1990, *110*, 290-305, (in Russian).

Seene T & K 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 of Sports Med, 1991, *12*, 204-207.

Seene T & M Umnova: 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. BAM 1992, *2*, 39-46.

Shepard R & P Gollnick: Oxygen Uptake of Rats at Different Work Intensities. Pflügers Arch, 1976, *362*, 219-222.

Sugiura T & N Murakami: Separation of Myosin Heavy Chain Isoforms in Rat Skeletal Muscles by Gradient Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. Biomedical Research, 1990, *11*, 87-91.

Sugiura T, A Morimoto & N Murakami: Effects of Endurance Training on Myosin Heavy-Chain Isoforms and Enzyme Activity in the Rat Diaphragm. Pflügers Arch, 1992, *421*, 77-81.

Wenger H, J Wilkinson, J Dallaire & T Nihei: 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.