DISSERTATIONES KINESIOLOGIAE UNIVERSITATIS TARTUENSIS
9

DIFFERENCE BETWEEN MYOSIN LIGHT AND HEAVY CHAIN ISOFORMS PATTERNS IN FAST- AND SLOW-TWITCH SKELETAL MUSCLE: EFFECT OF ENDURANCE TRAINING

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Dissertation is accepted for the commencement of the degree of Doctor of Philosophy in Exercise and Sport Sciences (Functional Morphology) on 28 April 2005 by the Council of the Faculty of Exercise and Sport Sciences, University of Tartu, Tartu, Estonia.

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Commencement: 21 June 2005

Publication of this dissertation is granted by the University of Tartu

ISBN 9949–11–075–0 (trükis)
ISBN 9949–11–076–9 (PDF)

Tartu Ülikooli Kirjastus
www.tyk.ee
Tellimus nr. 212

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LIST OF ORIGINAL PUBLICATIONS

The work based on the following publications:

1. **Seene T., Alev K., Kaasik P., Pehme A., Parring A.-M.** Endurance training: volume dependent adaptational changes in myosin. International Journal of Sports Medicine. 2005, 26: 1–7 (in press)

2. **Seene T., Alev K., Kaasik P., Pehme A**. Changes in fast-twitch muscle oxidative capacity and myosin isoforms modulation during endurance training. Journal of Sports Medicine and Physical Fitness. 2005 (accepted for publication)

3. **Järva, J., Alev, K., Seene, T.** The effect of autograftting on the myosin composition in skeletal muscle fibers. Muscle & Nerve. 1997, 718–727

ABBREVIATIONS

ATP	adenosine triphosphate
bw	body weight
Dia	diaphragm
EDL	*extensor digitorum longus* muscle
Endur	endurance-trained group
Exh	exhaustive exercise group
FM	fast isomyosin
FT	fast-twitch
G	glycolytic muscle fibers
HC	heavy chain
HSP	heat shock proteins
IEF	isoelectric focusing
Ks	fractional rate of protein synthesis
LC	light chain
MyHC	myosin heavy chain isoform (MyHC I, MyHC IIa, MyHC IId, MyHC IIb)
MyLC	myosin light chain isoform (MyLC 1_{slow}; MyLC $1_{fast;}$ MyLC $2_{slow;}$ MyLC $2_{fast;}$ MyLC 3_{fast} isoform)
mRNA	messenger ribonucleic acid
O	oxidative muscle fibers
OG	oxidative-glycolytic muscle fibers
Pla	*plantaris* muscle
PWC	physical working capacity
RT	resistance training
S1	subfragment 1
Sa, Sb	specific radioactivities of protein
SDS-PAGE	sodium dodecylsulphate polyacrylamide gel electrophoresis
Sol	*soleus* muscle
ST	slow-twitch
3-MeHis	3-Methylhistidine

INTRODUCTION

Muscle myosin accounts for 60 per cent of the total myofibrillar protein and is composed of two heavy and two pairs of myosin light chains that are associated with the head region of the myosin heavy chain (MyHC). In adult skeletal muscle different isoforms of myosin are synthesized according to the specialization into fast-twitch (FT) or slow-twitch (ST) muscles. Although some of the differences may be related to post-translational events, the analysis of amino acid sequence indicated that different myosin isoforms represent products of different genes (Salviati *et al*., 1983).

There are great differences between the maximum velocity of shortening not only in ST and FT muscles but also in FT muscles. Both MyHC content and the proportion of myosin light chain (MyLC), particularly alkali MyLC, are important determinants of unloaded shortening velocity (Sweeney *et al*., 1988; Bottinelli *et al*., 1994). Unfortunately, the role of MyLC isoforms in contractile machinery is not fully clear. Even the distribution of MyLC isoforms between ST and FT muscles as well as between FT muscles still shows considrable discrepancies. It has been proposed that the functional role of MyLC isoforms realizes in combination with certain MyHC isoforms (Stevens *et al.*, 2000; Wada and Pette, 1993), but the distribution of MyHC and MyLC isoforms in different skeletal muscles remains unclear. Exercise, depending on its character, induces differential expression of myosin protein isoforms in skeletal muscle (Baldwin and Haddad, 2002). Most exercise studies focus on the assessment of the composition of MyHC because of its regulatory role in myosin ATPase activity and, therefore, in velocity of muscle fibre shortening (Bottinelli, 2001; Gür *et al*., 2003). There are only a few studies that at the same time deal with the effect of exercise on the composition of MyHC and MyLC isoforms (Wahrmann *et al*., 2001; Wada *et al*., 2003). It is unclear how exercise with different intensity and duration affects the changes in the relative content of MyLC isoforms and their distribution in different muscles. The repetition regime in the exercise training protocol plays a very important role in the hypertrophy of muscle fibres. A large number of repetitions in resistance training (RT) did not cause any significant hypertrophy of muscle fibres (Campos *et al.*, 2002).

It is still not fully known how skeletal muscle responds to an increase in the mechanical load. It is known that compensatory hypertrophy is characterized by an increase in muscle mass, muscle protein content, and contractile force and by a shift from the fast-to-slow myosin isoform type in FT muscles. However, the exact mechanism of changed MyHC and MyLC isoforms during exercise training is poorly understood (Pehme *et al.*, 2004a, b). In comparison with MyHC isoforms much less is known about changes of MyLC isoforms during adaptation to exercise training. Mechanical activity with a low force causes

changes in skeletal myosin isoforms including the depressive effect on the contractile velocity of type IIB fibres (Wada *et al*., 2003).

Endurance training results in the regulation of enzyme systems of the Krebs cycle, electron transport chain, capillary supply, changes in key metabolic enzymes involved in fatty acid activation, and increased oxygen uptake (Hollozy and Booth, 1976; Hood, 2001). Endurance training does not result in hypertrophy of the skeletal muscle fibres involved in the exercise response because the level of force production is relatively low compared to their maximum force generation (Baldwin and Haddad, 2002).

Aerobic endurance training promotes a transition from type II to type I muscle fibres, which occurs at the expense of the type II fibre population (Thayer *et al.*, 2000). Myosin forms the backbone of the myofibrillar apparatus and serves as the regulator in the conversion of chemical energy into mechanical activity. There is a clear relationship between myosin isoforms and functional properties of the muscle. Maximum shortening velocity is higher in fast than in slow isoforms because the rate of actomyosin interaction is greater or because of the larger size of the step generated by single interaction (Bottinelli, 2001). It has also been shown that in rat MyHC I isoform propelled actin filaments at a lower speed than IIb isoform (Hook *et al*., 1999). Sports practice shows that both training volume and intensity of endurance athletes are increasing, which means that the role of FT muscles in endurance events is increasingly important. Endurance training causes transformation in the expression of MyHC isoforms with a more economical cross-bridge cycling kinetics, which means that intermediate-fastest forms are expressed in place of fastest MyHC isoforms, and the exercise becomes more economical to perform (Baldwin and Haddad, 2001). Unfortunately, little is known about changes in MyHC and MyLC turnover rate in FT muscles during endurance training, particularly in relation to an increase in oxidative capacity. It has been shown that the synthesis rate of MyHC I and IIa isoforms is faster than in others (Seene *et al*., 2004), and at least in pathological conditions the different MyHC isoforms show different sensitivity to proteinases (Seene *et al*., 2003). In the rat large variability of maximum shortening velocity in muscle fibres with the same MyHC content can account for the diverse composition of alkali MyLCs (Bottinelli *et al*., 1994; Bottinelli and Reggiani, 1995). MyHC IIb isoform seems to be more sensitive to alkali MyLC modulation in the rat (Bottinelli, 2001). It was found that the higher the MyLC 3_f isoform content the greater the maximum shortening velocity of the muscle fibres (Bottinelli *et al*., 1994; Bottinelli and Reggiani, 1995). Endurance training decreased MyLC 3_f isoform expression in some FT muscles, but in *plantaris* (Pla) muscle it increased (Wahrmann *et al*., 2001). Endurance training may exert a depressive effect on the contractile apparatus of type IIB fibres. This effect is caused by alterations in MyLC alkali complements within a given fibre type as well as by transitions in MyHC isoforms (Wada *et al*., 2003). Several studies show that type II fibres are recruited in the case of endurance training (Hoppler *et al*., 1985; Seene and

Umnova, 1992; Seene *et al*., 2004). Due to the diversity of molecular mechanisms in different fibre types of skeletal muscle, endurance training functions uniformly during intervention with regard to exercise (Seene *et al*., 2004). Changes in the ultrastructural level during endurance training support this standpoint (Seene and Umnova, 1992).

The isoforms of a particular protein are molecules with slight variations in amino acid composition, and they may alter either the structural, functional or enzymatic properties of that protein (Baldwin and Haddad, 2002). It seems that changes in myosin isoforms during endurance training might be qualified as qualitative remodelling of muscle by replacing one isoform by another, that is better suited for the adaptation to long-lasting low level of force – generating activity.

High-volume exercise training leads to chronic fatigue and decreased performance mainly because of lack of recovery. Body structures need time for regeneration after exercise. It is known that proteins of muscle contractile machinery regenerate slowly (Seene *et al*., 1999). The basic steps of the regeneration of myosin isoforms in skeletal muscle, particularly in FT muscle, have remained obscure. The regeneration of MyLC and MyHC isoforms in fastest muscles and their fibres is little understood. Both short-lasting vigorous and long-lasting endurance exercise cause destruction of the myofibrillar apparatus, and the regeneration of contractile proteins is a prerequisite for high performance.

REVIEW OF LITERATURE

1. Slow-twitch and fast-twitch skeletal muscles

Skeletal muscle can be characterized functionally as either ST or FT, and individual fibres can be classified on the basis of their contractile and metabolic properties as ST oxidative (O), FT oxidative-glycolytic (OG), and FT glycolytic (G). These fibre types contain unique MyHC isoforms as MyHC I (α, β), MyHC IIa, MyHC IIx, and MyHC IIb, respectively (Bär and Pette, 1988).

The skeletal muscle fibres reflect the diversity of mechanical tasks that specific muscles must perform. These tasks range from brief episodes of intense work requiring maximaum force generation to sustained tasks requiring endurance over extended periods (Karas and Williams, 1991). G fibres generate force rapidly, but show fatigue after a brief effort reflect the prevalence of fast myosin isoforms and the paucity of mitochondria. In contrast, O fibres express slow myosin isoforms in addition to fast myosin isoforms, they are rich in mitochondria and resist fatigue. These variations in the phenotype of skeletal muscle fibre that accompany specialization and embryonic development exibit substantial plasticity, even in adult muscles. Differentiated muscle fibres retain the potential to undergo large changes in the content of either contractile or metabolic components in response to external stimuli.

FT muscles are known to exhibit a three- to fourfold higher maximum unloaded shortening velocity compared with ST muscles (Schluter and Fitts, 1994). This difference is thought to be a manifestation of the higher myofibrillar ATPase activity of the FT muscle (Barany, 1967). An analysis of FT muscles *in situ* suggests that IIB fibres are faster than IIA fibres (Schluter and Fitts, 1994).

2. Structure of the myosin molecule

The native myosin exists as a complex molecule, composed of two HC and four LC. Each HC is associated with an alkali and regulatory LC, which both exist as fast ($MyLC_{fast}$) and slow ($MyLC_{slow}$) isoforms. Isoforms can be derived from the same gene through alternative splicing or from different genes of the same family (isogenes) (Bottinelli and Reggiani, 2000). Fast vertebrate muscles generally contain two types of fast alkali LC, LC 1_f and LC 3_f but only one type of the regulatory LC, LC 2_f. As there is a fast muscle with a pair of identical MyHC, and assuming that the regulatory MyLC exist only as LC 2_f homodimer, three light-chain-based isomyosins can be formed from three alkali LC combinations: LC 1_f homodimer (fast isomyosin FM3), LC 1_f/LC 3_f heterodimer (fast isomyosin FM2) and LC 3_f homodimer (fast isomyosin FM1) (Wada and Pette, 1993).

The existence of fast MyHC isoforms creates even a larger number of isomyosins. Isomyosin triplets were characterized for each of the three fast MyHC isoforms present in rat skeletal muscle, HC IIb, HC IId, and HC IIa. These fast isomyosins were designated according to their MyHC isoforms, respectively, as FM 1b, FM 2b, and FM 3b; FM 1d, FM 2d, and FM 3d; and FM 1a, FM 2a, and FM 3a (Table 1) (Pette, 2002). Variatious in the proportions of three isomyosins in muscles differing by their MyHC complement were in accordance with specific alkali LC distributions in the same muscles (Termin and Pette, 1991; Wada and Pette, 1993; Hämäläinen and Pette, 1995).

Table 1. Myosin light and heavy chain combinations of nine different fast isomyosin (Pette, 2002)

Isomyosin	**Light Chains (LC)**	**Heavy Chains (HC)**
FM1b	$(LC\ 3f)_2(LC\ 2f)_2$	$(HC\ IIb)_2$
FM2b	$(LC\ 3f)(LC\ 1f)(LC\ 2f)_2$	$(HC\ IIb)_2$
FM3b	$(LC\ 1f)_2(LC\ 2f)_2$	$(HC\ IIb)_2$
FM1d	$(LC\ 3f)_2(LC\ 2f)_2$	$(HC\ IId)_2$
FM2d	$(LC\ 3f)(LC\ 1f)(LC\ 2f)_2$	$(HC\ IId)_2$
FM3d	$(LC\ 1f)_2(LC\ 2f)_2$	$(HC\ IId)_2$
FM1a	$(LC\ 3f)_2(LC\ 2f)_2$	$(HC\ IIa)_2$
FM2a	$(LC\ 3f)(LC\ 1f)(LC\ 2f)_2$	$(HC\ IIa)_2$
FM3a	$(LC\ 1f)_2(LC\ 2f)_2$	$(HC\ IIa)_2$

Thus, the LC 1_f/LC 3_f ratio was remarkably higher in muscles with the predominance of HC IIa than in muscles with the predominance of either HC IIb or HC IId (Wada and Pette, 1993).

The suggestion that the affinity of the alkali LC 3_f with three fast HC isoforms decreases in the order HC IIb > HC IId >HC IIa was supported by results from a study on human muscle, relating variations in the LC 1_f/LC 3_f ratio to the relative volumes occupied by type IIA and type IIB fibres (Wada and Pette, 1993).

3. Role of myosin in skeletal muscle plasticity

The diversity of skeletal muscle fibres arises, in part, from the existence of polymorphism in myosins. During muscle development from embryonic till adult stage several myosin isoforms are sequentially expressed. Synthesis of

these isoforms is repressed at a given stage of development when they are replaced by the adult isoforms (Whalen *et al*., 1981).

Myosin plays an important role in dictating the funtional properties of skeletal muscle fibres. Myosin is known to exist as multiple isoforms in striated muscle as a result of polymorphic expression of both its heavy and light chain components (Diffee *et al.,* 1993). At present nine distinct isoforms of the MyHC have been identified in mammalian skeletal muscle, of which four are thought to be expressed in rodent limb muscles (Pette and Staron, 1990). In addition, at least three isoforms of the alkali LC and two isoforms of the regulatory LC have been identified in rat skeletal muscle. These MyHC and MyLC isoforms combine to form a number of native isomyosins (Tsika *et al*., 1987; Sweeney *et al*., 1988).

Changes in the expression of MyLC can be included in FT muscle by chronic low-frequency stimulation (Bär *et al*., 1989). Stimulation induces a sequential exchange of fast light-chain isoforms with their slow counterparts. In vitro experiments show, the alterations at the translation process change the pattern of specific mRNAs. Studies of co-existence of MyHC and MyLC isoforms in the same muscle fibre show that MyHC IIa is preferably associated with MyLC 1_f, whereas MyHC IIb is favourably associated with MyLC 3_f. Early studies of Pette *et al.* (1979) considered that variations in an amount of MyLC 3_f in single type II fibres reflected subpopulations of type II fibres. In regions of FT muscle composed largely of FT OG fibres, the relative proportion of the FM1 isoform is lower than in muscle regions that are largely composed of FT G fibres (Thomason *et al*., 1986). The *soleus* (Sol) muscle, which consists mainly of ST O and relatively few FT OG fibres, contains FM3 but not FM1 and FM2, and prolonged endurance training elicits a decrease in the ratio of MyLC 3_f to MyLC 1_f concurrently with the transformation from type MyHC IIb to MyHC IIa fibres (Wada and Pette, 1993).

The possible lower affinity of MyLC 3_f for MyHC IIa than MyHC IIb may be related to enhanced degradation of MyLC isoform (Seene *et al.,* 2003). In FT muscles stimulated with low frequency, MyLC 3_f is related to an increase in the free form on MyLC 3_f, concomitant with the replacement of MyHC IIb by MyHC IIa (Wada *et al.,* 1990). However, it is uncertain whether or not in all mammalian skeletal muscles such a relationship between MyLC and MyHC isoforms is applicable and maintained with increased or decreased contractile activities since the stimulation-induced changes in the MyLC pattern of rabbit FT muscles vary greatly from those of the rat (Bär *et al*., 1989).

In conclusion, the role of myosin isoforms is to support muscle plasticity that is regulated by characteristics of muscle kinetics and metabolism, first of all, related MyHC isoforms.

4. Role of myosin isoforms in shortening velocity of skeletal muscle

Force development and shortening in muscle result from interaction of myosin and actin. In vertebrate muscle fibres, the extent of interaction between actin and myosin is regulated by the concentration of sarcoplasmic Ca^{2+}. Ca^{2+} regulation of contraction in vertebrate striated muscle is mediated by troponin and tropomyosin whereas striated muscles of various invertebrate species are regulated by Ca^{2+} binding directly to myosin (Metzeger and Moss, 1992).

One component of the myosin molecule, the HC, determines the functional characteristics of the muscle fibre. In an adult these isoforms exist in four different varieties. These isoforms in human muscle include I, IIa, and IIx; in rodent muscle they include I, IIa, IId(x), and IIb. Type I fibres are also known as ST fibres, where as types IIA, IIX, and IIB are the FT fibres. The fibres are called slow and fast – the maximum contraction velocity of a single type I fibre is approximately one tenth that of type IIX fibre. The velocity of type IIA fibres is somewere between those of type I and type IIX. Although the maximum velocity of shortening correlates with both MyHC and alkali MyLC isoforms, several authors suggest an additional impact of regulatory MyLC (Larsson and Moss, 1993; Lowey *et al*., 1993b). In vitro motility assay indicates that the removal of a regulatory LC evokes a pronounced decline in the velocity of actin filaments on myosin (Lowey *et al*., 1993a; van Buren *et al*., 1994). The role of regulatory LC in shortening is supported by a single fibre study on human muscle (Larsson and Moss, 1993). Several studies suggest that alkali MyLCs also have a role in determining maximum velocity of shortening. Eddinger and Moss (1987) and Sweeney *et al*. (1988) reported that shortening velocity is higher in fibres that contain larger amounts of MyLC 3_f. Shortening velocity has been found to be proportional to MyLC 3_f content in single fast fibres studied by Greaser *et al.* (1988).

4.1. Molecular mechanism of muscle contraction by myosin light chains

Information from the crystal structure of the subfragment 1 (S1) of skeletal muscle myosin suggests that MyLC may stabilize the α-helical neck region of the myosin head (Patel *et al*., 1996) so that the force resulting from conformational changes near the active site is transmitted to the rod region of the molecule (Rayment *et al*., 1993). It has been shown that the removal of up to 50 per cent of the endogenous regulatory MyLC has little effect on either maximum Ca^{2+} -activated force or stiffness but significantly increases force and stiffness at submaximum levels of Ca^{2+} of skinned skeletal muscle fibres (Hofmann *et al*., 1990). In vitro force measurements (van Buren *et al*., 1994) confirmed the results of Hofmann et al (1990) that the removal of regulatory

MyLC has little effect on maximum force. Partial extraction of regulatory MyLC from skinned skeletal fibres indicated that it may be involved in conferring Ca^{2+} sensitivity on cross-bridge transitions that limit the rate of force development in steadily Ca^{2+}-activated fibres (Metzger and Moss, 1992). In fibres containing a mutant myosin regulatory LC having a defective divalent cation binding site, both maximum tension and stiffness were significantly reduced compared to control values (Diffee *et al*., 1995), suggesting that myosin heads containing regulatory LC that is unable to bind Ca^{2+} or Mg^{2+} have a reduced ability to form strongly bound cross bridges. These findings suggest that rather than playing a strictly structural role such as stabilizing the structure of the myosin head, regulatory MyLC may also serve a regulatory role, such as modulating the availability of cross bridges to bind to actin.

Two myosin LCs, one regulatory LC and one alkali LC, stabilize an extended α-helical segment in the hinge region of each MyHC. The MyLCs are necessary for full force development (Lowey and Trybus, 1995). Removal of either alkali or regulatory LC markedly reduces myosin velocity in an in vitro motility assay (Lowey *et al*., 1993a). Like the other contractile proteins, the alkali MyLCs represents a family of isoforms. Five alkali MyLC isoforms have been found in human skeletal muscle: embryonic alkali LC that is mainly expressed in embryonic muscle tissue, a major and minor slow isoform, and two fast isoforms alkali LC 1_f and LC 3_f, which are both encoded on the same gene. Alkali MyLC isoforms can bring forward different contractile properties on a given MyHC (Schiaffino and Reggiani, 1994). The alkali MyLCs are expressed in a fibre-type specific manner. In adult human skeletal muscle, FT fibres contain fast MyLC, whereas ST fibres contain alkali MyLC 1_s and variable amounts of the two fast alkali MyLCs (Jostarndt *et al.,* 1996).

Difficulties in establishing a relation between maximum shortening velocity and myosin isoforms are caused by the preferential association between MyLC 3_f and MyHC IIb and between MyLC 1_f and MyHC IIa (Wada and Pette, 1993). IIB fibres, in fact, could be faster than IIA fibres, not because they contained MyHC IIb, but because they contained larger amounts of MyLC 3_f and vice versa (Bottinelli *et al.,* 1994). To address this problem, it is necessary to relate maximum shortening velocity to the alkali MyLC ratio in single fibres containing only one known MyHC isoform. The only paper that followed this approach (Larsson and Moss, 1993) had to deal not only with the inability to separate all three fast MyHC isoforms but also with the problem that the human fast fibres showed coexistence of two of regulatory MyLC isoforms (MyLC 2_f and MyLC 2_s). Under these circumstances, no relationship between maximum shortening velocity and alkali MyLC ratio was found either in IIA or in IIB fibres. Undetected MyHC coexistence and variations in the alkali MyLC isoform ratio might form the basis for the large variability of maximum shortening velocity among fast fibres presumed to contain the same fast MyHC isoform (Bottinelli *et al.,* 1991, 1994; Larsson and Moss, 1993).

It seems established that the high variability of maximum shortening velocity in fibres with the same MyHC content can fully account for alkali MyLC composition, that is the higher the MyLC 3_f content the greater is the maximum shortening velocity (Bottinelli *et al*., 1994; Bottinelli and Reggiani, 1995). However, in human fibres, the same considerable variability in shortening velocity cannot be satisfactorily explained on the basis of MyLC isoform content. Alkali MyLC and regulatory MyLCs have no or hardly any role in explaining the variability in maximum shortening velocity independently of MyHC isoforms in normal physiological conditions. Like mice, the increase in shortening velocity in fibres with the same MyHC isoform content following endurance training (Widrick *et al.*, 1996) cannot be attributed to variations of alkali or regulatory MyLC content. So far the findings show that it is unlikely that the whole variability in shortening velocity observed in human muscle fibres depends on MyLC content. In this respect, it is interesting to note that the MyHC isoform, which seems more sensitive to alkali MyLC modulation in the rat, that is MyHC IIb, is not present in human skeletal muscle. This might partly explain why alkali LC has not been shown to significantly affect shortening velocity in human fibres (Bottinelli, 2001).

4.2. Myosin light chains in slow-twitch and fast-twitch muscle fibres

In IIA fibres the LC1/LC3 ratio is higher than in IIB fibres, but it is not entirely clear which subunits determine the contractile characteristics. Thus, although preliminary data showed differences in the unloaded shortening velocity of rabbit tibialis muscle fibres depending on whether they contain IIa or IIb MyHC isoform, these fibres also differed in their LC1/LC3 ratio (Sweeney *et al*., 1988). It has been shown that the unloaded shortening velocity of rabbit Sol muscle fibres containing both type I and type IIa MyHC isoforms was related to their ratio. Until now it has been impossible to ascribe a role only to the MyLC of mammalian skeletal muscle. Phosphorylation of the regulatory LC alters the force-calcium relationship but has no clear effect on shortening velocity (Sweeney *et al*., 1988; 1993). Most studies have examined the possible role of the two alkali LC heterogeneity of myosin by measuring ATPase activity in vitro. In view of these findings, one may assume that LC 3_f is characterized by a lower affinity for MyHC I isoform than for MyHC IIa, IId(x), and IIb isoforms. It is likely that in ST fibres are composed solely of MyHC I isoform but the majority of the translated MyLC 3_f exists in free form (Wada *et al.*, 1996).

4.3. Role of myosin light chain phosphorylation in muscle contraction

The MyLCs of striated muscles are not directly involved in the regulation of contraction. Regulatory LC is a component of myosin molecule that contains

sites suitable for phosphorylation and is able to modulate myosin-actin interaction (Perrie *et al*., 1973; Sweeney *et al*., 1993).

Phosphorylation of the regulatory MyLC by a MyLC kinase occurs in striated muscle. The consequences of phosphorylation are not well understood, but it may well be that its effect is to act as a molecular memory of contraction (Timson, 2003). Phosphorylation of the regulatory MyLC results in potentiation of contractile activity. The rate of force development is increased, tension is increased, and more force is generated at lower Ca^{2+} concentrations when the regulatory MyLC is phosphorylated. One possible explanation for this phenomenon is that repeated stimulation by elevated calcium ions concentrations not only results in repeated contraction but also in Ca^{2+} /-calmodulin dependent phosphorylation of the regulatory MyLC. This in turn leads to decreased calcium sensitivity of the contractile system, resulting in a myosin that responds more rapidly to an influx of calcium ions (Timson, 2003). This would have at least two potential benefits: the interval between contractions would be reduced and energy would be saved as less calcium ions would need to be pumped into sarcoplasm (Timson, 2003). Furthermore, if the phosphorylated state persisted after the cessation of contraction, it would serve as a memory of a recent contraction – permitting a rapid response to renewed activity (Stepkowski, 1995). The molecular mechanisms behind this decreased calcium sensitivity are unknown.

4.4. Myosin isoforms in pure and hybrid fibres

It is well known that pure fibre contain only one distinct MyHC isoform. Single fibre studies have allowed the elucidation of the metabolic heterogeneity and the adaptive potential of skeletal muscle fibres (Pette, 2001). Hybrid fibres co-express two or more MyHC isoforms (Pette and Staron, 1990). Thus, there is a continuum that spans between the very fast type IIB and slow type I fibres and in which pure fibres are bridged by hybrid fibres expressing their next-neighbour MyHC isoforms (Staron and Pette, 1993).

Although the MyLC does not seem to affect the actin-activated myosin ATPase activity, it has a significant impact on the shortening velocity (Lowey *et al*., 1993b). This influence is of interest in view of the existence of various isomyosins. The existence of two fast alkali MyLCs ($LC1_f$, $LC3_f$) generates three combinatorial patterns – a MyLC 1 homodimer, a MyLC 1/MyLC 3 heterodimer, and a MyLC 3 homodimer. Their combination with a pair of regulatory MyLC and a MyHC homodimer results in three electrophoretically distinct isomyosins.

The MyLC 3/MyLC 1 ratio was found to finely tune the shortening velocity, while coarse adjustment of shortening velocity is achieved by different MyHC isoforms. Obviously, the number of isomyosins increases in hybrid fibres especially by coexistence of fast and slow MyLC isoforms in combination with MyHC isoforms.

5. The effect of exercise on the contractile apparatus of the skeletal muscle

Different intensities of exercise training affect differently the metabolic profiles in single muscle fibre. Functional and structural changes of skeletal muscle fibre following exercise training appear gradually, being controlled by different factors (Takekura and Yoshioka, 1990; Seene and Umnova, 1992; Tiidus, 2000; 2001). It has been demonstrated that different exercise protocols result in selective activation of specific intracellular signalling pathways, which may determine the type of adaptation (Nader and Esser, 2001; Seene *et al*., 2004).

Resistance training results in increased muscle mass, fibre hypertrophy, and strength (McDonagh and Davis, 1987; Pehme *et al.*, 2004b). In contrast, endurance training results in increased mitochondrial density, capillary supply, changes in key metabolic enzymes, and increased maximal oxygen uptake (Hollozy and Booth, 1976). However, whereas the physiological and biochemical adaptations induced by these modes of exercise have been well characterized, the myosin isoforms, particularly MyLC underlying these specific adaptations, remain poorly defined. At present it is unclear how the muscle might sense the type of mechanical load. Skeletal muscle responds to exercise by specific qualitative and quantitative alterations in gene expression, provided that the stimuli are of sufficient magnitude and duration. In this process neuromuscular activity plays an important role, effecting fast to slow transitions in muscle fibre phenotypes. Changes in myofibrillar protein isoforms occur both in fast and slow muscles (Seene *et al.*, 2004). For mammalian limb muscles, the spectrum of MyHC isoforms spans from MyHC IIb on one end to MyHC Iβ on the other (Pette, 1998). The changes in MyHC isoform expression seem to occur in a sequential order. For example, the type IIB fibre does not switch directly from MyHC IIb to MyHC Iβ but will express MyHC IId(x) and MyHC IIa before expressing MyHC Iβ (Pette, 1998).

The sequence of the MyHC isoform transitions, originally deduced from studies on low-frequency stimulated muscle (Leeuw and Pette, 1993), corresponds to an order of changes in myofibrillar ATPase activity, unloaded shortening velocity, tension cost, and stretch-activation of pure and hybrid fibres in normal muscles (Galler *et al.*, 1997b; Hilber *et al*., 1997; Pette, 1998).

The myosin transition depends on the severity of exercise load. Exhaustive exercise causes damage of myofibrils (Seene *et al.*, 1999), decreases the rate of contractile proteins synthesis rate, and slows down turnover rate (Seene *et al.*, 1986; Seene *et al.*, 2004). The regeneration of MyHC and MyLC after exercise is poorly understood, however, it is known that MyHC and the actin turnover rate are faster in ST muscle fibres than in FT fibres (Seene *et al.*, 2004).

5.1. The effect of endurance training on the relative content of myosin isoforms of the skeletal muscle

More than a decade ago researchers showed fast to slow fibre type transition with endurance training in the rat (Schluter and Fitts, 1994). Baumann *et al*. (1997) showed an increase in slow MyLC and MyHC isoforms in histochemically typed IIA fibres after bicycle training.

It has been long known that the isozymes of myosin can be modified by exercise (Baldwin *et al.*, 1972; Rapp and Wicker, 1982).

In FT and mixed skeletal muscles endurance training exhibit an increase in the expression of MyLC 1_s, 1_f and 2_s and a decrease in MyLC 2_f and 3_f isoforms. A significant increase in MyHC I and IIa isoforms was observed in two out of the four fast muscles (Wahrmann *et al*., 2001). In the Sol muscle of endurance-trained rats the MyLC 1_s, 2_f and 3_f isoforms decreased and MyLC 2_s increased in comparison with the control group (Wahrmann *et al*., 2001). The expression of MyHC IIa in endurance-trained rats decreased and MyHC I isoform increased in comparison with the control group. All the muscles studied by Wahrmann, Winand, Rieu (2001) showed a significant shift towards slow myosin isoforms after endurance training. In Sol muscle regular endurance training increases the shortening velocity of type I fibre, this change is probably caused by an increase in myosin ATPase activity (Schluter and Fitts, 1994). It has not been conclusively established what caused the increased myosin ATPase activity, but in a small percentage it appears to be due to an increased content of fast MyLC and MyHC isoforms (Schluter and Fitts, 1994).

5.2. The effect of endurance training on muscle plasticity and the isoforms pattern of MyHC and MyLC

Skeletal muscle plasticity is based on the phenomenon that multigene and alternative transcript splicing create multiple, thick- and thin-filament protein isoforms covering a range of functional properties (Pette, 1998).

Previous experiments clearly demonstrated the capacity of skeletal muscle to adapt to endurance training by qualitative and quantitative changes in energy supply and protein catabolism, especially with regard to increased capacity of the oxidative metabolic pathways (Seene and Umnova, 1992; Seene *et al.*, 2004). Endurance training also evokes transitions in MyHC isoforms and MyHC-based fibre types. As studies of chronic low-frequency stimulation of rodent FT muscles show, transitions induced by increased contractile activities follow the order MyHC IIb $\rightarrow$ MyHC IId $\rightarrow$ MyHC IIa $\rightarrow$ MyHC I (Pette, 2001). Although endurance training results in a qualitatively similar transition, in most cases the changes are limited to the FT subtypes, consisting of a decrease in the faster MyHC IIb isoform with an concomitant increase in the slower MyHC IIa isoform (Allen *et al*., 2001).

Also, MyHC transitions within fast MyHC isoforms resulting from increased contractile activity have been shown to be accompanied by an increase in MyLC 1_f at the expense of MyLC 3_f (Wada *et al*., 1992). The type IIB fibres contain higher amounts of MyLC 3_f than type IID fibres, while the latter contain higher amounts of MyLC 3_f than type IIA fibres. These differences indicate distinct affinities of MyHC isoforms for fast alkali MyLC complement (Wada *et al.,* 2003). A study on in vivo synthesis rates of MyLC suggested that the activity-induced reduction in the MyLC 3_f content may be attributed, at least in part, to the decrement in MyHC IIb displaying a high affinity for MyLC 3_f (Kirschbaum *et al.,* 1989). A large scattering of the MyLC 3_f among the fibre types indicates that each fibre type is composed of identical fibres with regard to their specific MyHC complement but heterogeneous with regard to their composition of fast alkali MyLC. This raises the question of whether increased contractile activity, as it occurs in sustained exercise, elicits an alteration in the distribution of fast alkali MyLC, MyLC 1_f and MyLC 3_f, within a given fibre type together with MyHC transitions. The variability of shortening velocity observed in FT fibres is suggested to be attributable primarily to differences in the fast alkali MyLC complements, MyLC 1_f and MyLC 3_f, in each fibre type, because few FT fibres additionally contain slow regulatory MyLC isoforms. In fact, single fibre analysis demonstrated variations in the fraction of MyLC 3_f in each of three fast fibre types of the rat (Wada and Pette, 1993). The replacement of MyHC IIb by MyHC IIa may therefore result in an increase in the amounts of free form MyLC 3_f, which is more readily degraded than its bound form. It is accepted that the maximum shortening velocity correlates not only with MyHC but also with MyLC isoforms expressed in the fibres. The alterations in fast alkali MyLC in FT muscles suggests that endurance training may exert a depressive effect on the contractile velocity of type IIB fibres and that a training decreases the contractile velocity by alterations in the fast alkali MyLC patterns within a given fibre type besides the transition of MyHC-based fibre populations. The question regarding the effects of training on an alterations in the alkali MyLC pattern of isomyosins comprising MyHC IIa and MyHC IId remains unanswered (Wada *et al.,* 2003).

6. Mechanism of muscle fibre transition

When trying to determine the molecular basis of muscle fibre diversity and plasticity, it is important to understand mechanisms of fibre conversions (Pette, 2002). Unfortunately, it is unknown how muscle fibre types undergo conversion. For example we do not know whether the changes after exercise occur in the pre-existing fibres or in the new forming fibres, which then re-specify for the the given muscle tasks (Seene and Umnova, 1992; Seene *et al.*, 1999). Regeneration of the muscle fibres can be confirmed by observing the accumulation of mononuclear cells and centrally located myofibre nuclei, and by the presence of embryonic or fetal MyHCs in adult muscle. There is some evidence that the transformation of muscle types by chronic stimulation takes place in pre-existing muscle fibres. The existence of fibres that co-express MyHCs in patterns that follow the temporal transition of fibres during different kind of exercise would argue favourably for the conversion occuring in pre-existing and not regenerating myofibres (Seene *et al*., 2004). During chronic low- frequency stimulation that induces fast-to-slow transitions, up to 20 per cent of the fibres may undergo degeneration. Most of the degeneration occurs in the first days of stimulation and seems to affect most prominently the FG fibres (Baldwin and Haddad, 2002). Studies on chronically stimulated muscles of the rat showed that MyHC transitions in the oder HCIIb $\rightarrow$ HCIId $\rightarrow$ HCIIa were accompanied by decreases in both synthesis and relative concentration of LC 3_f (Pette and Staron, 1993). Although exercise training causes transition of MyHC and MyLC isoforms in different muscle fibres, it is not clear how stable these changes can be. For example, it has been shown that detraining and hindlimb suspension reverses the changes in MyHC and MyLC isoforms (Bottinelli, 2001).

7. Regeneration capability of contractile proteins

All muscle proteins are in balance between synthesis and the degradation process. In conditions of muscle activity degradation of proteins increased already during longlasting exercise and continued after that. It was shown long ago that the rate of protein synthesis increases when the degradation intensity decreases (Seene *et al*., 1986). The muscle protein synthesis rate of contractile proteins myosin and actin is low to other compared myofibrillar proteins (Baldwin and Haddad, 2002). The degradation mechanisms of the contractile proteins are poorly studied, but the ATP dependent ubiquitin – protesome pathway is likely involved in this process (Solomon *et al.*, 1998). It has been shown that serine proteinases participate in the degradation process of myosin, particularly MyHC (Seene *et al.*, 2003, 2004). Unfortunately, both the synthesis and degradation of MyLC are poorly characterized, and the role of MyLCs in

the process of regeneration of myofibrillar apparatus is unknown. Our previous studies showed that the turnover rates of MyHC I and MyHC IIa isoforms are faster than MyHC IIb (Seene *et al.,* 2004). Vigorous short-lasting exercise increased the synthesis rate of MyHC IId isoform and continous activity MyHC I isoform in skeletal muscle (Pehme *et al.,* 2004b). As the MyLC turned over faster than MyHC (Seene *et al.,* 1986), one might expect the MyHC and MyLC play different roles in the regeneration process of the myofibrillar apparatus.

7.1. Grafting as a model of muscle regeneration

The grafting of different skeletal muscles is a promising approach to study the regeneration of skeletal muscle fibres (Carlson, 1986). The regeneration of contractile proteins is a long process, and the grafting of muscles is the fastest way to receive information about the basic steps of regeneration To monitor the turnover rate of certain proteins is an indirect possibility to follow the process of regeneration as changes in the turnover of myosin during and after exrercise is regarded as an adaptation of the contractile apparatus to increased functional activity. The latter may provide a rapid and effective means for the redistribution of amino acids into new proteins as they are required because amino acids derived from protein breakdown are preferentially reincorporated into the newly synthesized protein (Righetti *et al.,* 1971).

Studies of standard and nerve-intact *extensor digitorum longus* (EDL) muscle grafts in rats have shown that the functional characteristics of the repaired grafts depend on whether they were standard or nerve-intact grafts. The nerve-intact EDL grafts do not have any functional deficits. However, in standard grafts, maximum isometric tetanic tension is only about 50 per cent of that of the non-grafted value. (Galler *et al*., 1994). Faulkner and Carlson (1986) showed that at week two standard grafts were more resistant to fatigue than the muscles of non-grafted controls, whereas the fatigue ability of nerve-intact grafts was different from the control value. By week eight, both grafts were more resistant to fatigue than the control muscles (Faulkner and Carlson, 1985).

Muscle grafting is associated with significant metabolic rearrangement. The EDL standard grafts have been characterized by reduced glycose utilization (Wineniger *et al.,* 1991). This change is compensated by increased succinic dehydrogenase activity, which shows that regenerating FT muscles become more like oxidative muscle (compensate their decreased ability to initiate glycolysis by becoming more oxidative) (Gorin *et al.,* 1989). The MyHC content of the Sol muscles is also altered; it changes from the mixed, slow and fast to the homogenous slow type during regeneration (Davis *et al*., 1989). It is proposed that this change is caused by the cycles of denervation and reinnervation, rather than the cycle of degeneration and regeneration (Davis *et al*., 1989). In the latter studies degeneration and regeneration were induced by the injection of snake venom into the Sol muscle that causes muscle necrosis

and temporal loss of innervation. In this model, however, muscle is completely regenerated at approximately 30 days after injection (Wahlen *et al.*, 1990). Using muscle grafting, complete recovery of the graft in rats takes about 60 days (Carlson, 1986). Reinnervation of regenerating muscle fibres exerts a repressive effect on the expression of embryonic MyHC isoforms but not on neonatal or adult fast myosin isoforms (Cerny and Bandman, 1987). In contrast, denervation causes the reexpression of embryonic MyHC isoforms in adult muscle fibres (Jakubiec-Puka *et al.*, 1990).

8. Unsolved problems

Although the function of MyHC isoforms in the myofibrillar apparatus of FT and ST muscles is almost known, the role of MyLC isoforms has remained obsure, particularly during adaptation to different mechanical loading. Removal of alkali and regulatory MyLCs from myosin decreases the velocity of filament sliding by ten times, without a significant decrease in ATPase activity (Lowey *et al*., 1993a), difference between FT muscles in dependence of their oxidative potential is still unclear. Expression of MyHC and MyLC isoforms in FT muscles with different oxidative potential is also unclear. Adaptation of muscle to intensive and vigorus short-lasting exercise training has been shown to involve expression of slower isoforms and depress fastest one (Allen *et al*., 2001; Demirel *et al.*, 1999). Unfortunately, the mode of expression of MyLC isoforms during exercise training is unclear not only in FT and ST muscles but also in FT muscles. Although MyLCs are not essential for enzymatic activity in skeletal muscle, their interaction with MyHC isoforms may play an important role in the conversion of chemical energy into movement (Bottinelli, 2001).

Parallel changes of MyHC and MyLC isoforms in ST and FT muscles, and in FT muscles with different oxidative potential, occurring in response to the resistance and endurance training, may point to functional significance of each isoform in the process of adaptation of contractile apparatus to muscular activity. These isoforms may have different sensitivity to the degradation and have a different synthesis rate. Exhaustive exercise causes destruction in contractile machinery (Seene and Umnova, 1992; Seene *et al.*, 1999) and decreases the turnover rate of contractile proteins (Seene *et al*., 2004). Unfortunately, the information about regeneration of myosin isoforms after exercise is still lacking. As the process of regeneration of myofibrillar proteins is time-related, the grafting model of muscle regeneration may provide valuable information about this process, particularly regarding the coexistence of MyLC 3_f and fast MyHC IIb isoforms in FT muscles.

AIMS OF THE STUDY

The aim of the present study was to show differences in the relative content of MyLC isoforms in fast-twitch and slow-twitch skeletal muscles and between fast-twitch muscles, similarities with MyHC isoforms, and relations during adaptation to the different volume of endurance training and recovery after exercise.

The specific aims of the study were as follows:

1. To investigate the relative content of MyLC isoforms in fast-twitch and slow-twitch muscles and changes in the turnover rate of contractile proteins during adaptation to exercise training.
2. To find differences in expression between MyLC and MyHC isoforms in fast-twitch and slow-twitch muscles, to establish changes in the coexistence of MyHC IIa and MyLC 1_f and MyHC IIb and MyLC 3_f isoforms in fast-twitch muscles during adaptation to exercise.
3. To establish the effect of exhaustive exercise on the MyHC and MyLC isoforms pattern during regeneration in fast-twitch muscles.

MATERIALS AND METHODS

1. Animals

The animals used were 16–22 weeks old (n = 80); in autografting experiment 12–14 weeks old (n = 30) rats of the Wistar strain (National Laboratory Animals Centre, Kuopio, Finland). Rats were randomly divided into groups. All the animals were housed in identical environmental conditions in polycarbonate type III cages, at 21ºC, two per cage at 12/12 hrs light/dark period. They received diet (SDS-RM1 (C) 3/8, Witham, Essex, England) and water *ad libitum.* Rats were weighed at the beginning and at the end of the experiment. The animals were used in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and under control by the Committee of Laboratory Animal Science, University of Tartu.

2. Endurance training

After a brief 5-day acclimation that consisted of treadmill running for 5–10 min, the rats were subjected to run at a speed of 35 m/min. The rats were running 5 days per week; the training volume was increased moderately during 6 weeks from 10 min to 60 min per day. In the exhaustive group training volume was increased faster than in the endurance training group and reached 2 h 12 min per day in the 4th week. Work per training session, per week, and total work was expressed in kJ as well as in physical working capacity (PWC) 24 h after the last training session. The power of work did not change during the training period. For the determination of PWC, the rats were running on the horizontal treadmill at a speed of 35 m/min until exhaustion. The volume of work done by the animals and the power of exercise was calculated as described earlier (Kaasik *et al*., 1996).

Another exhausted group swam in water 33 ± 1°C, 125 cm^2 water surface area per rat in a pool of 40 cm depth for 2 weeks. On the first day for 5 h and from the 4th day on 10 h daily. The rats were used to determine the turnover rate of MyHC and MyLC.

3. Autografting of skeletal muscle

Autografts were prepared from EDL muscles using nerve-intact models (Carlson *et al*., 1981). The EDL muscle from the left limb was completely removed from the leg and grafted back to its original bed by suturing the distal and proximal tendons. No attempt was made at neural or vascular anastomoses.

During nerve-intact grafting the motor nerve branches to the muscle were left intact. The EDL muscle from the contralateral limb served as control. 10, 30, and 60 days after grafting muscles were removed, weighed, and frozen in liquid nitrogen, and analysed for the relative content of MyLC and MyHC isoforms.

4. Measurement of muscle strength

The forelimb and hindlimb grip strength was measured weekly with a Grip Strength Meter 0167-004L (Columbus Instruments, Columbus, USA) and expressed as N/100 g bw.

5. Preparation of muscle sample

L-[4.5^{3}H] leucine (170 Ci/mol) was infused intraperitoneally of 1.0 ml for 2 h in the exhaustive swimming experiment 6 h, 100 μCi per 100 g bw; 250 μCi per 100 g bw, immediately after the last exercise, 10 h and 22 h after the last exercise, in order to determine the dynamics of the protein synthesis rate. The incorporated radioactivity was measured in a liquid scintillation counter.

10 % SDS-PAGE gel electrophoresis was carried out (Porzio and Pearson, 1977), and the identified MyHC bands were sliced and dissolved in hydrogen peroxide at 50 ºC overnight, and radioactivity was determined.

Prior to being sacrificed, the animals were anaesthetized by intraperitoneal injection of ketamin (Calysol, Gedeon Richter A.O. Budapest, Hungary) and diazepam (Lab Renaudin, France). The EDL muscle then the Pla muscle, Sol muscle, diaphragm (Dia), and the *gastrocnemius* muscle were quickly removed, trimmed clean of visible fat and connective tissue, weighed, frozen, and stored in liquid nitrogen pending further processing. Three samples were taken from each muscle.

6. Separation of total muscle protein

The minced muscle samples were homogenized in a buffer containing: 50 mM KCl, 10 mM K_2 HPO_4, 1 mM EGTA, 1 mM $MgCl_2$, and 1 mM dithiothreitol, at pH 7.0, and analysed as total protein fraction. The total muscle homogenate was dissolved in 0.3 M NaOH and was analysed for radioactivity and protein.

7. Separation of myofibrillar protein

Frozen muscles were thawed on ice and washed with five volumes 20 mM NaCl, 5 mM Na_2HPO_4, 1 mM EGTA (pH 6.5). Myofibrillar protein was extracted with three volumes 100 mM $Na_4P_2O_7$, 5 mM EGTA, 1 mM dithiothreitol (pH 8.5) and centrifuged after 30 min of gentle shaking, diluted with one volume glycerol and stored at –80°C. Protein was assayed by using the technique described by Bradford (1976).

8. Fractional synthesis rate of muscle proteins

The fractional rate of protein synthesis Ks (expressed by the percentage of the protein synthesized per day) in each fraction was then calculated from the following relationship: Ks = 100 x Sb/Sa x *t*, where Sa and Sb are specific radioactivities of the total muscle cell protein and protein-bound leucine and *t* is the incorporation time in days (Sugden and Fuller, 1991).

9. Turnover rate of MyHC and MyLC

In order to investigate the turnover rate of contractile proteins in endurance trained rats, the double isotope method as descriebed by us previously was used (Seene, Umnova, 1992). The relative turnover rate of the protein fraction was estimated from $^{3}H/^{14}C$ ratios. Protein with a higher turnover rate would have a greater $^{3}H/^{14}C$ ratio.

10. One-dimensional electrophoresis

The isoform composition of MyLC (Fig. 1C) and MyHC (Fig. 1A) was determined by SDS-PAGE (Fig. 1). MyHC isoforms were separated by the previously described method (Hämäläinen and Pette, 1996) with the 7.2% separating gel. Electrophoresis was run for 24 hrs at a low temperature with 120 V (constant voltage). Gels were silver-stained by the method of Oakley *et al.*, 1980.

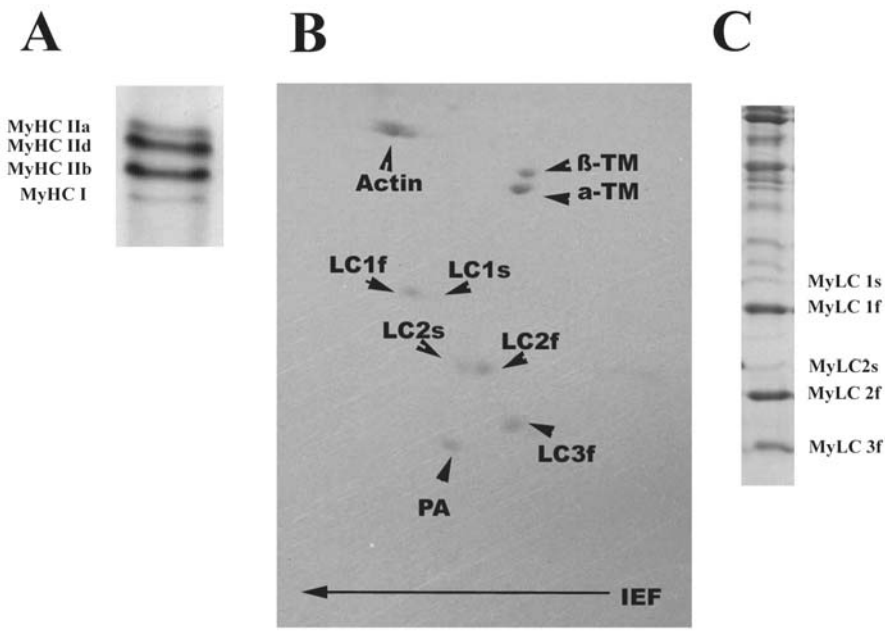

Figure 1. One- and two-dimensional gel electrophoresis of MyHC and MyLC isoforms from fast-twitch muscle
A – one-dimensional separation of MyHC isoforms
B – two-dimensional separation of MyLC isoforms
C – one-dimensional separation of MyLC isoforms

The MyLC isoforms were analysed by 12.5% one-dimensional SDS-PAGE according to Laemmli (1970), except that the glycerol content in the separating gel was 10%. Electrophoresis was performed at a constant current (30 mA) and stopped when the dye front reached the bottom of the gels using the vertical slab gel system (Protean II Xi Bio-Rad). The gels were stained with Coomassie Brilliant Blue R-250.

Identification of MyLC and MyHC isoforms was based on the migration rate. MyLC and MyHC isoforms were quantified densitometrically by the computer-based image analysis system and software (Image Master 1D, Version 4.0, Amersham Pharmacia Biotech, Newcastle upon Tyne, England).

11. Two-dimensional electrophoresis

Myofibrillar proteins were separated by two-dimensional gel electrophoresis according to O'Farrell (1975). Electrophoresis in the first dimension was performed on glass capillaries by using 1.6% (pH 5.0–7.0) and 0.4% (pH 3.0–

10.0) ampholines (Servalyt) in 4.2% polyacrylamide gel. Electrophoresis was first run for 30 min at 100 V, then 1 hr at 300 V and 1 hr at 400 V. Separation in the second dimension was carried out at 70 mA with 1.5 mm thick 15% separating gel and 3% stacking gel (Wada and Pette, 1993). The gels were stained with Coomassie Brilliant Blue R 250. After destaining, the spots corresponding to MyLC isoforms (Fig 1B) were excised, placed into tubes containing 1.5 ml 25% (vol/vol) pyridine, and incubated overnight. This allowed the eluation of the bound dye to determine relative protein amounts. The eluted dye was measured spectrophotometrically at 605 nm (Fenner *et al*., 1975). Two-dimensional electrophoresis was used for the identification of different MyLC isoforms.

12. Other analyses

Cytochromes aa_3 were measured by the method described by Schollmeyer and Klingenberg (1970). Serine proteinase separation and activity measurement was provided by Dahlmann *et al.*, 1981.

The degradation rate of MyHC and MyLC isoforms was measured with the incubation of myofibrils with alkaline proteinase at 37 °C during 20 min. The degradation rate of myosin isoforms was expressed as percentage decay of radioactive label from isoforms.

13. Estimation of 3-methylhistidine in skeletal muscle and urine

The 3-methylhistidine (3-MeHis) in skeletal muscle and urine was used as an indicator of the degradation of contractile proteins. The determination was performed as described previously (Seene and Alev, 1985). The total muscle protein was hydrolyzed in 6 M HCl for 20 h at 110°C in vacuum-sealed flasks. HCl was removed by evaporation, and the hydrolysate was dissolved in 0.2 M pyridine to achieve a concentration of 10–20 mg/ml. 3-MeHis in the urine and muscle tissue was estimated with HPLC (Seene and Alev, 1985).

14. Statistics

Means and standard errors of means were calculated from individual values by standard procedures. The data were analysed by SAS procedures, using the analysis of variance (ANOVA) and the Pearson correlation coefficients, and partial correlation coefficients were calculated. The differences were considered significant at $p<0.05$.

RESULTS

1. Difference in the relative content of MyLC and MyHC isoforms between fast-twitch and slow-twitch muscles

The relative content of MyLC isoforms in FT muscles (EDL and Pla) is significantly different from that of ST muscles (Sol) (Fig. 2). The difference in the relative content of MyLC isoforms between two FT muscles exists only in MyLC 1_s and 2_s isoforms (Fig. 2). Differences in the relative content of MyHC (Fig. 3) isoforms between two FT muscles are considerably bigger than those of MyLC isoforms (Fig. 2). The comparison of the relative content of MyLC and MyHC isoforms between ST and FT muscles revealed a good agreement between MyLC and MyHC (Fig. 2 and 3). Fig. 2 shows that the relative content of MyLC isoforms in Dia muscle remains between that of FT and ST muscles.

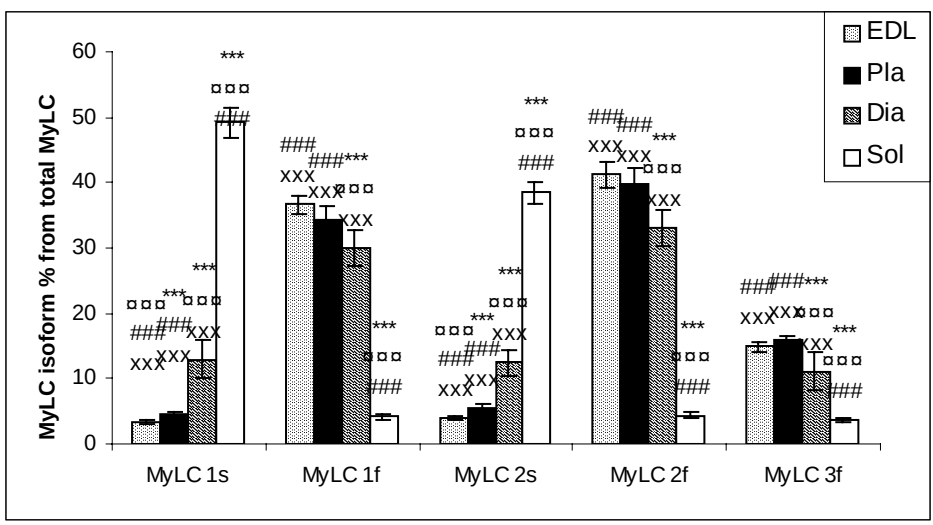

Figure 2. Relative content of MyLC isoforms in different muscles
EDL – e*xtensor digitorum longus* muscle; Pla – *plantaris* muscle
Dia – diaphragm; Sol – *soleus* muscle
MyLC 1s, MyLC 1f, MyLC 2s, MyLC 2f, MyLC 3f – myosin light chain isoforms
* – $p<0.05$
*** – $p<0.001$
} in comparison with the corresponding EDL isoform
¤¤¤ – $p<0.001$ in comparison with the corresponding Pla isoform
– $p<0.001$ in comparison with the corresponding Dia isoform
xxx – $p<0.001$ in comparison with the corresponding Sol isoform

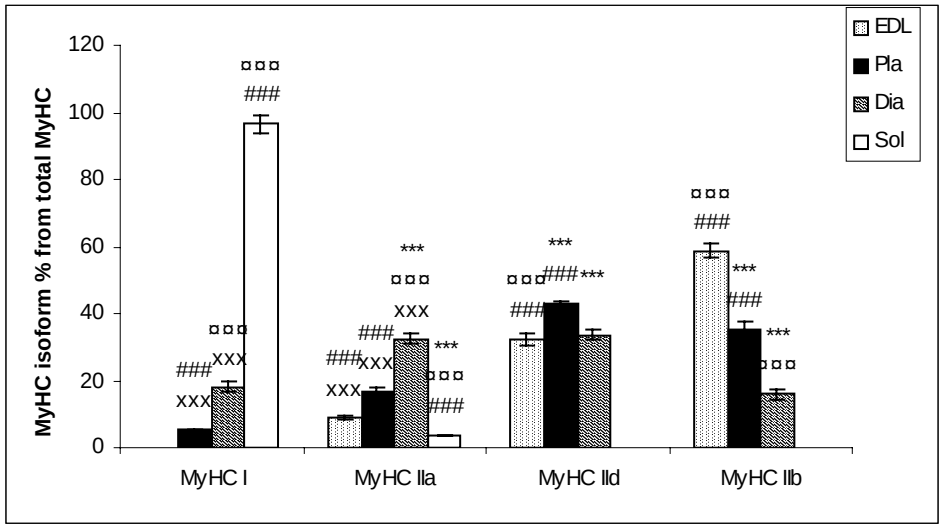

Figure 3. Relative content of MyHC isoforms in different muscles
EDL – *extensor digitorum longus* muscle; Pla – *plantaris* muscle; Dia – diaphragm; Sol – *soleus* muscle
MyHC I, MyHC IIa, MyHC IId, MyHC IIb – myosin heavy chain isoforms
*** – $p<0.001$ in comparison with the corresponding EDL isoform
¤¤¤ – $p<0.001$ in comparison with the corresponding Pla isoform
– $p<0.001$ in comparison with the corresponding Dia isoform
xxx – $p<0.001$ in comparison with the corresponding Sol isoform

The histogram of the MyLC isoforms in the Sol muscle (Fig. 4) shows that the distribution of MyLC slow isoforms is significantly wider than that of fast isoforms, which is not typical of the FT muscles.

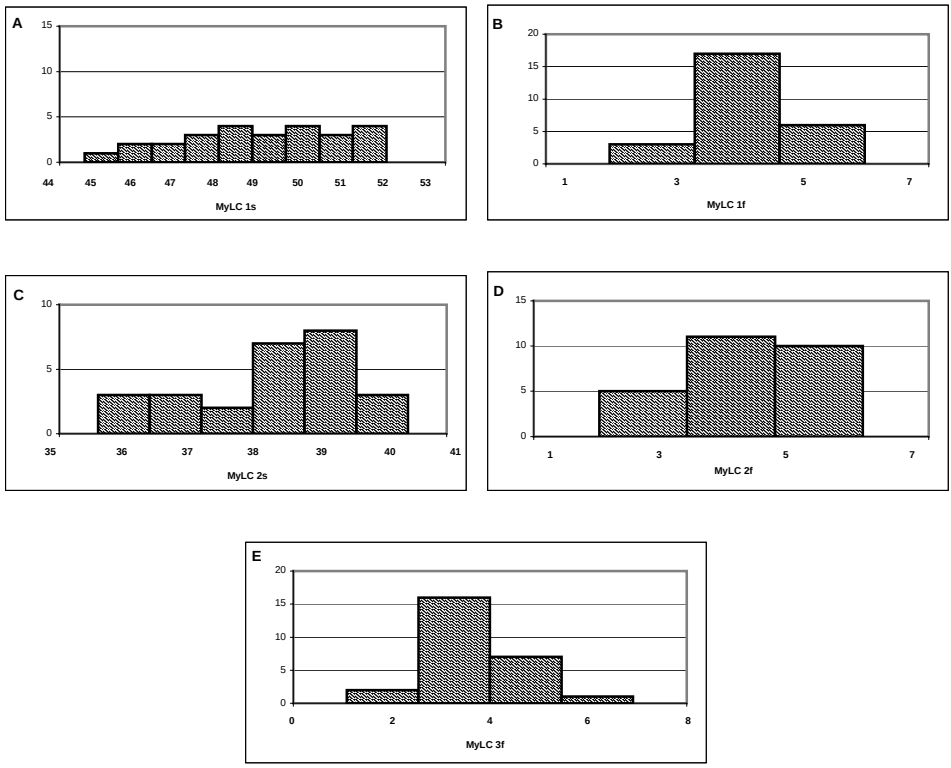

Figure 4. Histogram of MyLC isoforms in *soleus* muscle
A – MyLC 1s isoform; B – MyLC 1f isoform; C – MyLC 2s isoform; D – MyLC 2f isoform; E – MyLC 3f isoform

2. Difference in the relative content of MyLC and MyHC isoforms between fast-twitch muscles

The relative content of MyLC isoforms between two FT muscles does not follow the logic of MyHC. There are many more similarities between the relative content of MyLC and MyHC isoforms when we sum up both MyLC and MyHC into fast and slow isoforms (Table 2). There are no significant changes between alkali and regulatory MyLC isoforms between Pla (54.7%; 45.3% respectively) and EDL muscles (54.8%; 45.2%). The difference between Pla muscle and EDL muscle in MyHC IIa isoform was 7.59% and 2.24% in MyLC 1_f isoforms. The corresponding differences between these muscles in MyHC IIb and MyLC 3_f isoforms were 23.31% and 0.92%. The relative content

of MyHC IIb isoform in the EDL muscle is about twice as high as that in the Pla muscle, but there is no difference between the relative content of MyLC 3_f isoform between these muscles.

Table 2. Comparison of the relative content of MyHC and MyLC fast and slow isoforms in different muscles

Myosin isoforms	Muscles			
	Sol	Dia	Pla	EDL
MyHC fast (%) (IIa+IId+IIb)	3.56±0.12 *** ¤¤¤ ###	81.90±1.46 *** ¤¤¤ xxx	94.92±2.09 *** xxx ###	100.00±1.91 ¤¤¤ xxx ###
MyLC fast (%) (1f+2f+3f)	12.35±1.36 *** ¤¤¤ ###	74.02±2.84 *** ¤¤¤ xxx	89.95±2.81 ** xxx ###	92.70±3.04 ¤¤¤ xxx ###
MyHC slow (%) (I)	96.44±2.77 ¤¤¤ ###	18.10±1.44 ¤¤¤ xxx	5.08±0.17 xxx ###	–
MyLC slow (%) (1s+2s)	87.66±1.96 *** ¤¤¤ ###	25.39±2.49 *** ¤¤¤ xxx	10.09±1.63 *** xxx ###	7.30±1.01 ¤¤¤ xxx ###

EDL – *extensor digitorum longus* muscle; Pla – *plantaris* muscle; Sol – *soleus* muscle; Dia – diaphragm

** – $p<0.01$
*** – $p<0.001$
} in comparison with the corresponding EDL isoform

¤¤¤ – $p<0.001$ in comparison with the corresponding Pla isoforms

xxx – $p<0.001$ in comparison with the corresponding Sol isoforms

– $p<0.001$ in comparison with the corresponding Dia isoforms

3. Effect of exercise on the isoforms pattern of MyLC and MyHC in fast-twitch and slow-twitch muscles

Endurance training caused a significant decrease in the relative content of MyLC 2_s isoform in EDL muscle muscle and MyLC 1_s isoform in the Pla muscle (Fig. 5A, B).

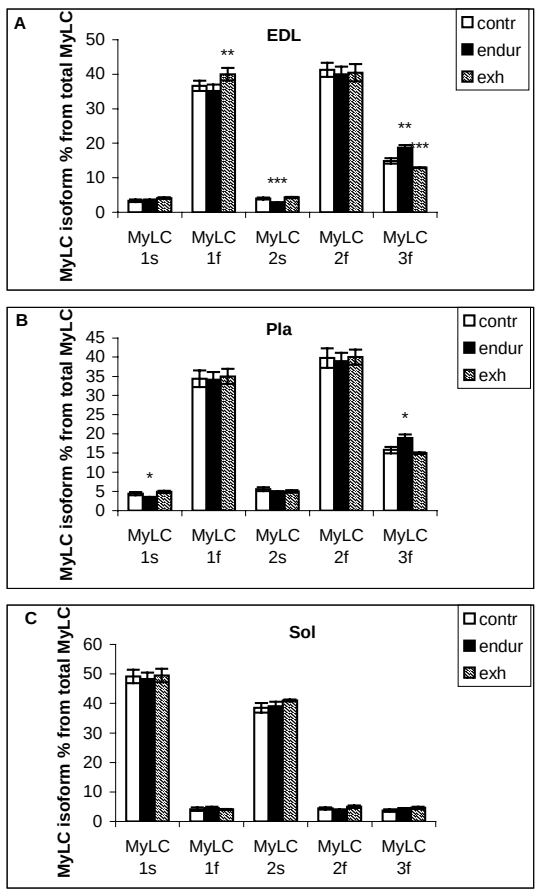

Figure 5. Effect of the volume of endurance training on the relative content of MyLC isoforms in fast-twitch and slow-twitch muscles

contr – control group; endur – endurance-trained group

exh – exhaustive exercise group

EDL – *extensor digitorum longus* muscle; Pla – *plantaris* muscle; Dia – diaphragm; Sol – *soleus* muscle

MyLC 1s, MyLC 1f, MyLC 2s, MyLC 2f, MyLC 3f – myosin light chain isoforms

* – $p<0.05$
** – $p<0.01$
*** – $p<0.001$

} in comparison with the corresponding MyLC isoform of control group

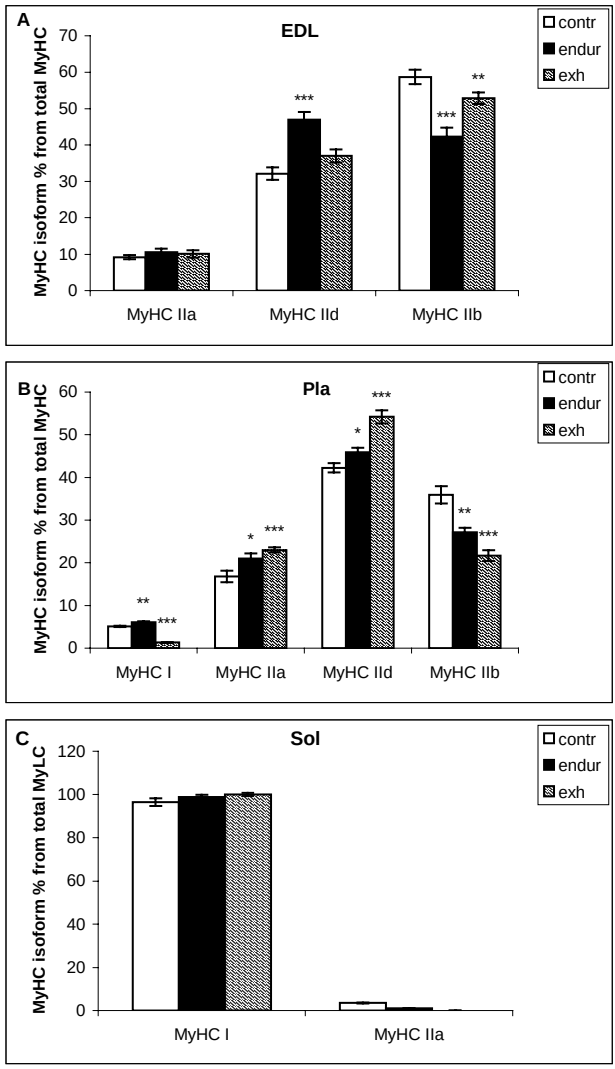

Figure 6. Effect of the volume of endurance training on the relative content of MyHC isoforms in fast-twitch and slow-twitch muscles

contr – control group
endur – endurance-trained group
exh – exhaustive exercise group
EDL – e*xtensor digitorum longus* muscle; Pla – *plantaris* muscle
Dia – diaphragm; Sol – *soleus* muscle
MyHC I, MyHC IIa, MyHC IId, MyHC IIb – myosin heavy chain isoforms
* – $p<0.05$
** – $p<0.01$
*** – $p<0.001$
} in comparison with the corresponding MyHC isoform of control group

The relative content of MyLC 3_f isoforms increased in both FT muscles during endurance training. The MyLC 3/MyLC 2 ratio increased during endurance training in FT and ST muscles. In EDL muscle the ratio increased 33 ± 6.4% ($p<0.001$) and in Pla muscle 23 ± 4.6% ($p<0.001$) during endurance training. In Sol muscle the ratio increased by 10 ± 1.2% ($p<0.001$). In both FT muscles the relative content of the MyHC IIb isoform decreased, and the MyHC IId isoform increased during endurance training (Fig. 6A, B). The relative content of MyHC IIa isoform increased during endurance training in the Pla muscle. The relative content of MyHC I isoform increased during endurance training regimes in the Pla muscle, but it did not change significantly in the Sol muscle (Fig. 6C). In the Sol muscle the relative content of the MyHC IIa decreased during endurance training (Fig. 6C).

An excessive increase in the training volume, which leads to exhaustion, decreased the relative content of the MyHC IIb isoform in FT muscles. In the Pla muscle, where the oxidative potential is higher than in the EDL muscle, the relative content of the MyHC I isoform decreases and MyHC IIa and isoforms increase (Fig. 6A, B). In the ST muscle exhaustive exercise increased the MyHC I isoform and decreased the relative content of the MyHC IIa isoform (Fig. 6C). In the ST and FT Pla muscles exhaustive exercise did not cause any significant changes in the relative content of MyLC isoforms (Fig. 5B, C). However, in the FT EDL muscle the relative content of the MyLC 1_f isoform increased and the MyLC 3_f isoform decreased (Fig. 5A). The experiment shows that myosin isoforms react differently to the increase of changes in exercise volume not only between ST and FT muscles but also between two FT muscles. In the Sol muscle an increase in the training volume had a negative correlation with the relative content of MyHC IIa isoforms ($r=-0.742$).

4. Effect of endurance training on the oxidative capacity of skeletal muscle

Average PWC in endurance-trained rats was 11.73 ± 0.55 kJ/100 g bw and 0.48 ± 0.02 kJ/100 g bw in untrained rats. The forelimb and hindlimb grip strength did not change significantly after six weeks of endurance training. The average forelimb grip strength was 3.81 ± 0.18 in the control group and 4.00 ± 0.18 N/100 g bw in the endurance group. The hindlimb corresponding indicators were 6.25 ± 0.36 and 6.61 ± 0.38 N/100 g bw. 3-MeHis was used as the indicator of the degradation rate of contractile protein (Table 3). Table 3 shows that in the Pla muscle oxidative capacity is about 2.4 times higher than in the EDL muscle. Endurance training increased the oxidative capacity about 16% in the Pla muscle and about 12% in the EDL muscle.

Table 3. Characteristics of the oxidative capacity of skeletal muscle and the degradation of myofibrillar protein

Measures	Control group (n = 8)	Endurance-trained group (n = 8)
Body weight (g)	259 ± 7	248 ± 6
Muscle mass (g/rat)	113.96 ± 3.14	113.12 ± 3.11
Myofibrillar protein (g/rat)	15.27 ± 0.72	15.84 ± 0.78
Daily excretion of 3-MeHis (%)	2.06 ± 0.15	3.24 ± 0.29**
Plantaris muscle weight (mg)	249.22 ± 7.08	250.61 ± 7.45
Cytochrome aa_3 (nmol/muscle)	3.61 ± 0.18	4.22 ± 0.20*
Cytochrome aa_3 (nmol/mg muscle)	0.0145 ± 0.0005	0.0168 ± 0.0006*
Extensor digitorum longus muscle weight (mg)	113.45 ± 4.26	114.26 ± 4.61
Cytochrome aa_3 (nmol/muscle)	1.49 ± 0.06	1.69 ± 0.07*
Cytochrome aa_3 (nmol/mg muscle)	0.0131 ± 0.0004 #	0.0147 ± 0.0004* #

n = number of animals per group; 3-MeHis – 3 Methylhistidine
Daily excretion of 3-MeHis was used as an indicator of contractile protein degradation.
Cytochrome aa_3 was used as an indicator of muscle oxidative capacity
* – $p<0.05$
** – $p<0.01$
} in comparison with the control group
\# – $p< 0.05$
\#\# – $p< 0.01$
} in comparison with the EDL

5. Effect of endurance training on the relative content of MyLC and MyHC isoforms

The Pla muscle is a fast muscle with ~75% of its MyHC pool existing as MyHC IIb and MyHC IId. In the Pla muscle of untrained animals MyHC IIb composed ~36% of total MyHC, this percentage declined during endurance training ~22%. At the same time training promoted a significant increase (~33%) in MyHC IIa (Fig. 7). The reduction in MyHC IIB isoform (~30%) in the EDL muscle was accompained by a concomitant increase in the percentage of MyHC IIb (~46%) and MyHC IIa (~19%) compared with the control group (Fig. 7).

Unlike to MyHC isoforms, the relative content of the predominat MyLC isoforms did not change significantly in the studied FT muscles (Fig. 8). MyLC 3_f isoform increased in the Pla muscle as well as in the EDL muscle (Fig. 8). In comparison with the control group MyLC $1_f/1_s$ isoform ratio and MyLC $2_f/2_s$ ratio increased ($p<0.001$) in endurance-trained rats in the Pla muscle. In the EDL muscle only the MyLC $2_f/2_s$ ratio increased ($p<0.01$) in endurance-trained group. MyLC $3_f/2_s + 2_f$ and MyLC $3_f/1_f + 3_f$ isoforms ratio increased ($p<0.001$) in both studied muscles during endurance training.

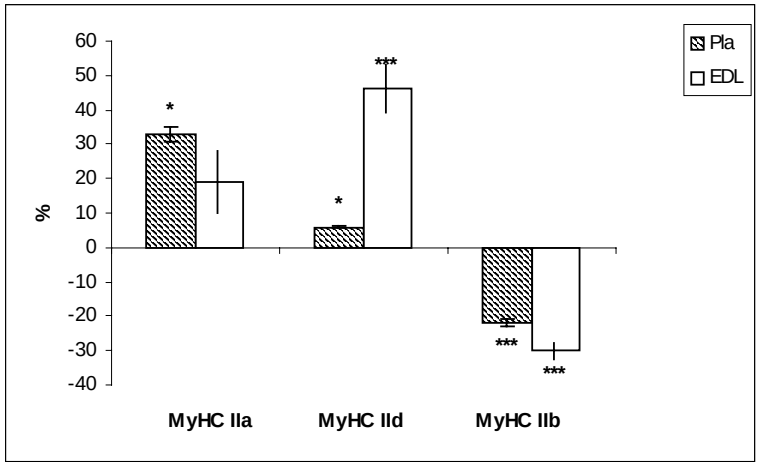

Figure 7. Percentile changes in the relative content of fast MyHC isoforms after endurance training in *plantaris* and *extensor digitorum longus* muscle compared with the controls

Pla – *plantaris* muscle; EDL – *extensor digitorum longus* muscle

MyHC IIa, MyHC IId, MyHC IIb – myosin heavy chain isoforms

* – $p<0.05$

*** – $p<0.001$ } in comparison with the control group

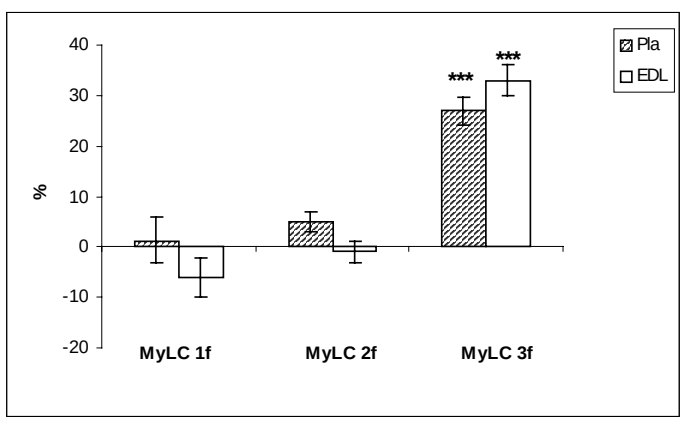

Figure 8. Percentile changes in the relative content of fast MyLC isoforms after endurance training in *plantaris* and *extensor digitorum longus* muscle compared with the controls

Pla – *plantaris* muscle; EDL – *extensor digitorum longus* muscle

MyLC 1s, MyLC 1f, MyLC 2s, MyLC 2f, MyLC 3f – myosin light chain isoforms

*** – $p<0.001$ in comparison with the control group

The relative content of MyLC alkali isoforms and MyLC regulatory isoforms did not change significantly in the studied muscles during endurance training.

6. Effect of endurance training on the degradation of MyHC isoforms

More intensive liberation of the radioactive label in endurance-trained rats from all types of MyHC isoforms during incubation with proteinase shows that the degradation of MyHC is increasing (Fig. 9).

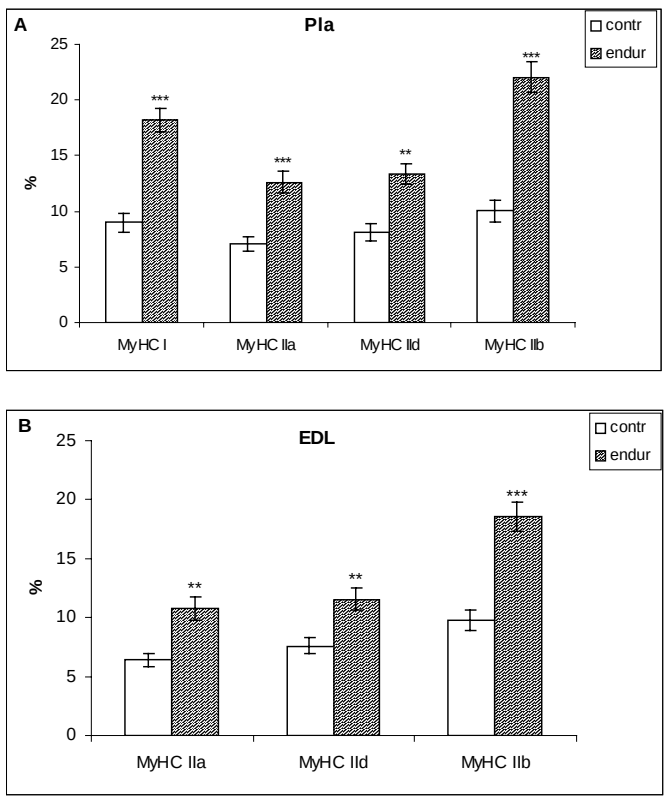

Figure 9. Changes in the degradation rate MyHC isoforms after endurance training

contr – control group
endur – endurance-trained group
Pla – *plantaris* muscle; EDL – *extensor digitorum longus* muscle
Myofibrils (5 mg/ml) were incubated with alkaline proteinase (0.7 U/ml) at 37°C during 20 min. The degradation rate of MyHC isoforms expressed as a percentage decay of radioactive from MyHC isoforms.
MyHC I, MyHC IIa, MyHC IId, MyHC IIb – myosin heavy chain isoforms
** – $p<0.01$
*** – $p<0.001$
} in comparison with the corresponding control group

Figure 9 also shows that MyHC I and IIb isoforms are more sensitive to alkaline proteinase than other isoforms in endurance-trained rats. MyHC isoforms, except IIb isoform, turned over faster after six weeks of endurance training in both studied muscles (Fig. 10). At the same time the turnover rate of MyLC isoforms did not change significantly (Fig. 11).

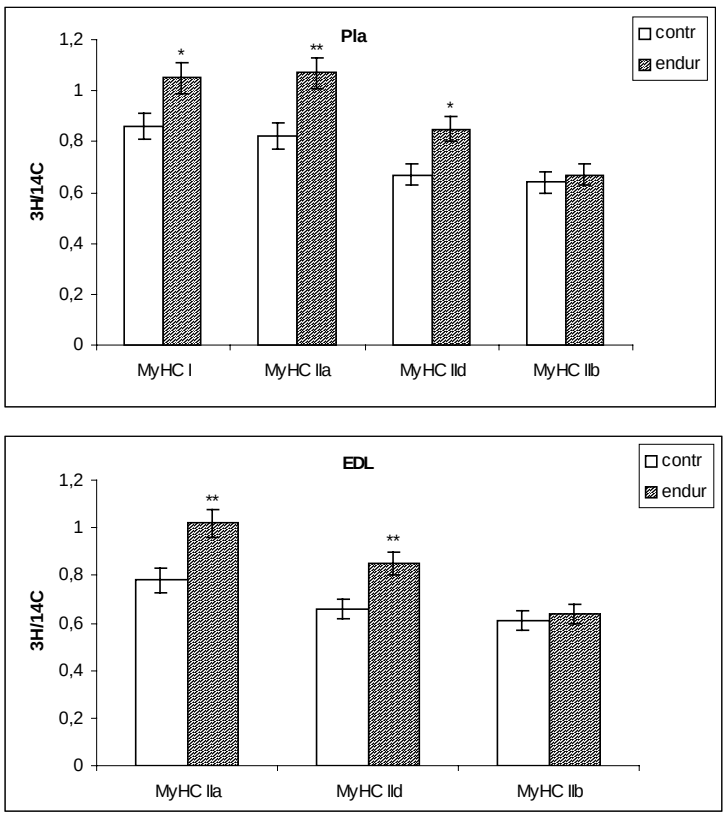

Figure 10. Effect of endurance training on the turnover rate of MyHC isoforms in fast-twitch muscles

contr – control group

endur – endurance-trained group

Pla – *plantaris* muscle; EDL – e*xtensor digitorum longus* muscle

MyHC I, MyHC IIa, MyHC IId, MyHC IIb – myosin heavy chain isoforms

The relative turnover rate of the protein fraction was estimated from the $^{3}H/^{14}C$ ratios. The $^{3}H/^{14}C$ ratios were expected for the same protein turnover rates, to be the same. Protein with a more rapid turnover rate would have a greater $^{3}H/^{14}C$ ratio.

* – $p<0.05$
** – $p<0.01$
} in comparison with the control group

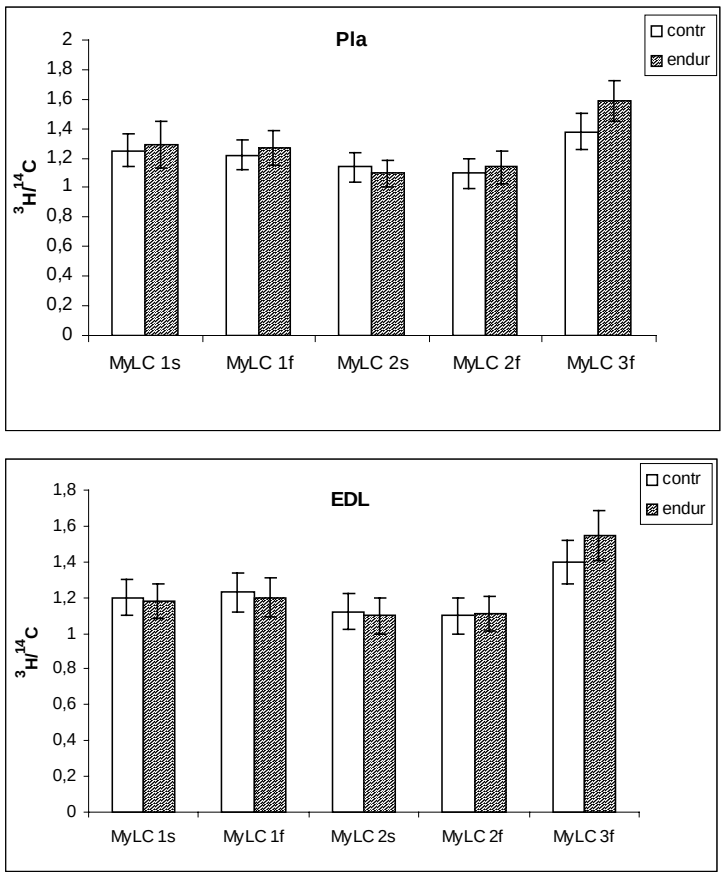

Figure 11. Effect of endurance training on the turnover rate of MyLC isoforms in fast-twitch muscles

contr – control group

endur – endurance-trained group

Pla – *plantaris* muscle; EDL – *extensor digitorum longus* muscle

MyLC 1s, MyLC 1f, MyLC 2s, MyLC 2f, MyLC 3f – myosin light chain isoforms

The relative turnover rate of the protein fraction was estimated from the $^3H/^{14}C$ ratios. The $^3H/^{14}C$ ratios were expected for the same protein turnover rates, to be same. Protein with a more rapid turnover rate would have a greater $^3H/^{14}C$ ratio.

Endurance training increased the oxidative capacity of FT muscles and changed the relative content of MyHC and MyLC isoforms. As Figure 12 shows, endurance training did not reveal any changes in the relative content of MyHC IIa and MyLC 1_f isoforms in the EDL muscle. During exhaustive exercise (excessive increase in the training volume), an increase in the relative content of the MyHC IIa isoform caused an increase in the relative content of MyLC 1_f isoform.

Exhaustive exercise decreased MyHC IIb and MyLC 3_f isoforms relative content. This experiment shows that during trainable endurance training there is no correlation between changes in the coexistence of MyHC IIa and MyLC 1_f isoforms and MyHC IIb and MyLC 3_f isoforms in the FT muscle. On the other hand, exhaustive exercise, which leads to the extreme situation in skeletal muscle, reveals a good correlation between MyHC IIa and MyLC 1_f isoforms and MyHC IIb and MyLC 3_f isoforms. This experiment proves that MyLC isoforms react to the changes in the relative content of MyHC isoforms in extreme situations. It means that in conditions that need maximum regulation of the contractile apparatus, MyHC and MyLC isoforms function synchronously.

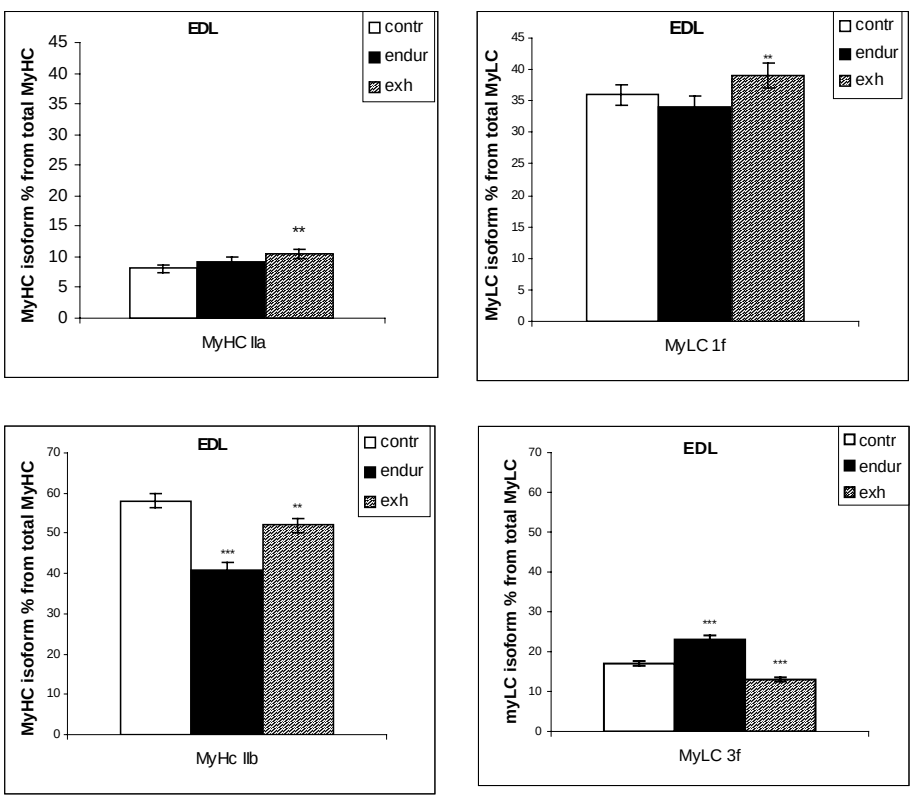

Figure 12. The effect of the volume of endurance training on the coexistence of MyHC IIa and MyHC 1_f, MyHC IIB and MyLC 3_f isoforms in *extensor digitorum longus* muscle

contr – control group
endur – endurance-trained group
exh – exhaustive training
MyHC IIa, MyHC IIb – myosin heavy chain isoforms
MyLC 1f, MyLC 3f – myosin light chain isoforms
** – $p<0.01$
*** – $p<0.001$
} in comparison with the control group

7. Recovery of the MyHC and MyLC isoforms after exhaustive exercise

During long exhaustive exercise the turnover rates of MyHC and MyLC 1, 2 and 3 isoforms were taken as indicators of myosin recovery. An increase in the turnover rate of certain myosin fractions shows the state of recovery. As seen in Figure 13, MyHC turnover in the FT muscle increased with time after exhaustive exercise. 24 h after exercise revealed no differences between the turnover rate of MyHC in comparison with the control group. The turnover rate of MyLC 2 and MyLC 3 increased 12 h after exhaustive exercise and remained on the control group level immediately and 24 hours after exercise (Fig. 13).

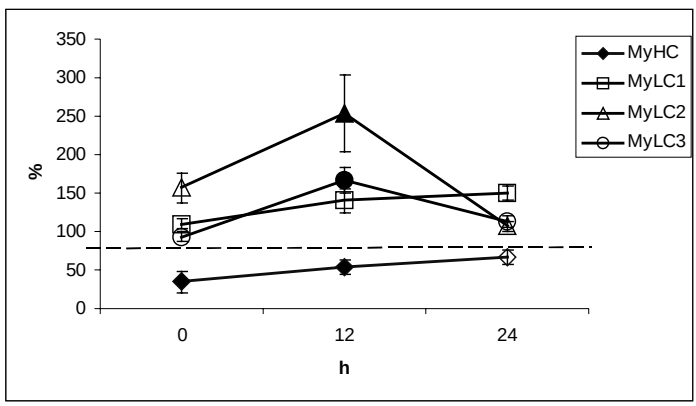

Figure 13. **Dynamics of percentile changes in turnover rate of MyHC and MyLC in the *gastocnemius* muscle after exhaustive swimming**
100% – control group level
0 – immediately after swimming
12, and 24 hours after swimming
MyHC – myosin heavy chain
MyLC 1, 2, 3 – myosin light chains
Filled symbols – $p<0.05$ in comparison with the control group

8. Regeneration dynamics of MyHC IIb and MyLC 3_{fast} isoforms

It is known that a decrease in muscle mass reflects a decrease in muscle fibre number and atrophy of muscle fibres. In nerve-intact grafts the mass of grafted muscles is not significantly different from the control muscle (Table 4). As noted, earlier regeneration of FT muscles is slower than that of ST muscles. FT muscles with a higher oxidative potential regenerate faster. Figure 14A and B show that MyHC IIb isoform regeneration is relatively slow in comparison with the MyLC 3_f isoform. It was previously mentioned that these isoforms have a

high affinity to each other. In comparison with the control level MyHC IIb isoform the relative content is significantly lower even 60 days after grafting; the relative content of the MyLC 3_f isoform remained lower only during the first 30 days after grafting (Fig. 14B).

Table 4. Changes in the muscle mass and relative content of MyLC 3 [MyLC 3/(MyLC 1_f + MyLC 3)] in the control and grafted *extensor digitorum longus* muscle

Days after grafting	Muscle mass (mg)		MyLC relative content	
	Control	Grafted	Control	Grafted
10	90 ± 2.54	81.7 ± 3.35	0.397 ± 0.094	0.379 ± 0.025
30	103 ± 3.5	93.2 ± 4.02	0.435 ± 0.021	0.247 ± 0.018*
60	110.8 ± 8.96	105 ± 6.43	0.483 ± 0.025	0.479 ± 0.014

* – p<0.02 in comparison with the control group

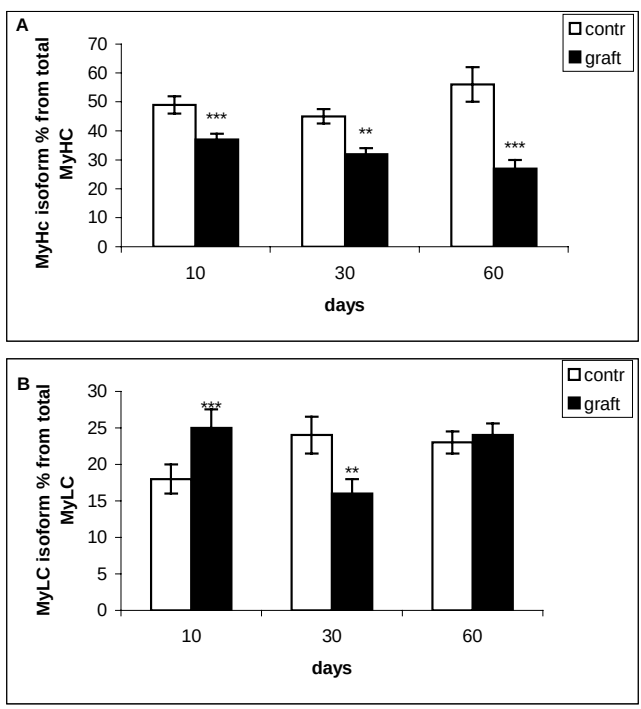

Figure 14. **Dynamics of changes in the relative content of MyHC IIb (A) and MyLC 3_f (B) isoforms after grafting of the *extensor digitorum longus* muscle**

contr – control group; graft – grafting group

** – p<0.01
*** – p<0.001
} in comparison with the control group

DISCUSSION

1. Myosin isoforms as the basis of muscle kinetics

1.1. Differences in molecular motors in different types of muscles

The skeletal muscle fibres are classified according to their metabolic and kinetic criteria. Mammalian skeletal muscles are composed of ST type I and FT type II fibres, based on different MyHC complement.

Adult mammalian skeletal muscle fibres are composed of at least four different MyHC; the heart muscle contains α- and β-MyHC. β-MyHC isoforms were found also in ST skeletal muscles, and developing muscles have been shown to express several embryonic MyHC isoforms, as well as a neonatal myosin (Pette and Staron, 1990). In addition to MyHC isoforms, numerous variants of slow and fast MyLC have also been described (Pette and Staron, 1990). Since the myosin molecule consists of two HC and two pairs of LC, a large number of hybrid myosins is potentially possible. It has generally been assumed that the velocity of shortening of a given muscle or fibre correlates with the enzymatic activity of the composition of its myosin isoforms. However, with the exception of slow versus fast skeletal myosin, and α versus β cardiac myosin, no important differences in ATPase activity have been demonstrated among the many developmental MyHC isoforms or MyLC – based isoforms (Lowey *et al*., 1993a).

Studies revealed different FM1/FM2/FM3 ratios of MyHC IIb-, MyHC IId, and MyHC IIa-based isomyosins, suggesting that these differences relate to varying LC 1_f/LC 3_f ratios (Wada *et al.*, 1995). Thus, the fraction of LC 3_f, that is, the (LC 3_f) /(LC 1_f + LC 3_f) ratio, was the highest in type IIB fibres and lowest in type IIA fibres. Type IID fibres exhibited an intermediate position (Wada *et al.*, 1995). In vitro motility assay showed clear distinctions in function between the alkali MyLC isoforms and between embryonic and adult fast skeletal muscle myosin (Lowey *et al.*, 1993b). The findings support the hypothesis that the diversity in shortening velocities is closely related to the type of MyLC and MyHC isoform present in a muscle fibre. Although widely studied, it is not well known what role MyLC isoforms play in muscle contraction and how the combination of MyHC and MyLC determines the muscle kinetics.

1.2. Parallel changes in the isoform patterns of MyLC and MyHC and muscle function

The past two decades have shown that among multiple isoforms of muscle proteins MyHC and MyLC play an important role in muscle function. Wahrmann *et al.* (2001) quantified changes in MyLC isoforms in different muscles during exercise. They claim that in order to understand better the function of

MyLC in skeletal muscle, it is necessary to study changes in MyLC alongside the quantification of MyHC under the same conditions. The above-mentioned standpoint is supported by studies where during muscle atrophy an increase of MyLC 1_f and 2_f isoforms was found to occur alongside an increase of MyHC IIb isoform relative content (Diffee *et al*., 1991). Hayashibara and Miyanishi (1994) showed that a decrease in the relative content of MyLC 1_f isoform serves as an indicator of the slowdown of muscle contraction. Our study supports this standpoint as the relative content of MyLC 1_f isoform decreases in muscles in the following direction: EDL → Pla → Dia → Sol.

There is also a relation between the fastest isoform of MyHC and the relative content of MyLC 3_f isoform and muscle contraction speed (Bottinelli, 2001). Some studies shown that there exists a positive correlation between muscle contraction speed and the ratio of MyLC 3_f /MyLC 1_f isoforms (Bottinelli *et al.*, 1994; Sweeney *et al*., 1988). On the other hand, some researchers have denied it (Larsson and Moss, 1993). Our study shows that the above-mentioned relation does exist between the FT and ST muscles but not between two FT muscles (EDL and Pla) because the MyLC3f /MyLC1f+MyLC3f ratio increased in both muscles. Apparently, there exist higher affinities of the MyLC 3_f isoform with MyHC IIb and IId isoforms (Stevens *et al*., 2000; Wada and Pette, 1993). The present study shows that there are correlations between MyHC IIb, IId, and the relative content of MyLC 3_f isoforms between the ST and FT muscles. No correlation was found between the ratio of MyLC 1_f /MyLC 3_f isoforms and the relative content of MyHC IIb and IIa isoforms in skeletal muscle. In muscles with the predominant fastest isoform of MyHC, the ratio of MyLC 1_f /MyLC 3_f isoforms is lower than in muscles with the predominant MyHC IIa isoform (Wada and Pette, 1993).

The low affinity of MyLC 3_f and higher affinity of MyLC 1_f isoform with MyHC IIa isoform show differences between myosin isoforms in FT muscles (Wada *et al.*, 1990). The present study shows that certain relations coexist with MyHC and MyLC isoforms in FT and ST muscles. The histogram of the distribution of MyLC isoforms in different muscles shows that in the ST muscle the distribution of predominat slow isoforms is wider than in fast isoforms. Wide distribution of predominat isoforms may show the physiological role and adaptability of MyLC isoforms in skeletal muscle to the everyday activity as the ST muscles are participate in the process of slow movements and keeping static positions. Wide distribution of MyLC slow isoforms in the Sol muscle may show the adaptational pecularities of muscle via these isoforms.

1.3. Effect of exercise training on the pattern of myosin isoforms

Adaptation to the exercise training different by duration and intensity revealed some similarities in changes of the relative content of MyHC isoforms in FT muscles during both type of exercise. Thus, endurance and resistance training

cause a decrease in the MyHC IIb isoform although with bigger changes during endurance training than during resistance training in FT muscles. The relative content of MyHC IId isoforms also increases with both training regimes in FT muscles (Pehme *et al*., 2004b; Seene *et al*., 2004). At the same time the relative content of MyLC 3_f isoforms is increased or has a tendency to increase in these muscles during both training regimes. There is a discrepancy between changes in the fastest isoform of MyHC and the relative content of MyLC 3_f isoform during the adaptation to exercise training. As the total content of MyLC fast isoforms did not change at the same time and the relative content of MyHC IId isoform increased and MyLC 3_f isoforms have an affinity to bind with the MyHC IId isoform, there is no contradiction from the standpoint of the stoichiometry of the myosin molecule. In the ST muscle there is full agreement between the increase of MyHC IIa isoform and the relative content of MyLC 2_f and 3_f isoforms during resistance training (our unpublished results). Endurance training leads to a decrease in the relative content of the MyHC IIa isoform in the Sol muscle, but MyLC fast isoforms reveal no significant changes. At the same time the ratio of MyLC 3 and MyLC 2 isoforms show an increase during both types of exercise training. It is interesting that the above-mentioned ratio is twice higher during resistance training. It was shown that the MyLC 3/MyLC 2 ratio increased during unloading and during the increase in muscle shortening velocity (Widrick and Fitts, 1997). An increase in the MyLC 3/MyLC 2 ratio in both FT and ST muscles during endurance and resistance training disagrees with the results of Williamson *et al.* (2001) but shows that mechanical loading leads to changes in MyLC isoforms in skeletal muscle. The present study revealed some discrepancy between the modulation of MyHC and MyLC isoforms in different muscles during the adaptation to the long-lasting low-intensity exercise. A change in MyHC and MyLC isoforms in FT and ST muscles in response to exercise training provides no reason for lessening the role of MyLC isoforms in skeletal muscle function. As the function of myofibrillar proteins depends on the contractile, regulatory, and minor proteins, the role of MyLC isoforms in ST and FT muscles needs further investigation in the context of change of other myofibrillar proteins.

2. Effect of endurance training on the character of skeletal muscle kinetics

It has been demonstrated that aerobic endurance training leads to metabolic (Viru and Viru, 2000) and structural changes not only in ST but also in FT muscle fibres (Hoppler *et al*., 1985; Seene and Umnova, 1992). In the present study changes in the expression of MyHC and MyLC isoforms are in good agreement with the above-mentioned findings. Endurance training increased the above-mentioned capacity in the Pla muscle by 16% and in the EDL muscle by

12%. At the same time in both studied muscles the expression of MyHC isoforms undergoes change. It has been shown that exercise training in FT fibres causes up-regulation of MyHC IIa and IIx isoforms, whereas the MyHC IIb is down-regulated (Demirel *et al*., 1999). Multidirectional changes have also been revealed in the relative contents of MyHC isoforms in different FT muscles if the training volume is increased (Seene *et al.*, 2004). These changes might be related to the differences in oxidative capacity of muscles (Seene and Umnova, 1992). Until now the question to what extent gene expression of MyHC isoforms is due to genetic predisposition or to the specificity of training remains unanswered (Baldwin and Haddad, 2001).

As a myosin molecule is formed by MyHC and MyLC isoforms and the functional significance of both in muscle contraction has been proved, it should be more useful to study the expression of MyHC and MyLC isoform in parallel during endurance exercise training in order to obtain information about the adaptative pecularities in the contractile machinery. It is unlikely that the whole variability in contractile machinery during endurance training depends on the content of MyHC isoforms. MyHC isoforms are the main determinants of muscle contraction, but the findings of this study prove taht there is also some ground to believe that MyLC has a functional significance in the process of adaptation of sarcomeric proteins to the long-lasting exercise.

The decrease of MyHC IIb isoforms during endurance training in both studied FT muscles does not necessarily show that in these muscles contractile properties change towards ST as the relative content of MyHC IIa and IId isoforms increases. Rather, these changes show that it is more economical for the FT muscles to perform the exercise. The decrease in slow isoforms both in alkali and regulatory MyLC during endurance training and the increase in MyLC 3_f isoforms in FT muscles is at first glance not in logical agreement with changes in the MyHC isoform pattern. However, the stoichiometry of these subunits and their association with each other do not change (MyHC IIb decreased but IId increased and MyLC 3_f is associated with both, IIb and IId MyHC to form myosin molecules). This shows that there are no definite adaptational borders between MyHC and MyLC isoforms in FT muscles to the long-lasting endurance exercise. The adaptation process consists of both changes in MyHC and MyLC during aerobic endurance training and MyLC may also be associated with transformation of muscle function (Seene *et al*., 2004).

The present study shows that the slowest and fastest MyHC isoforms have a higher sensitivity to the process of degradation. In myopathic FT muscles MyHC IIb isoform is more sensitive to the serine proteinase than other isoforms (Seene *et al.*, 2003). This experiment reveals that both the slowest and fastest MyHC isoforms are the most sensitive to the activity of proteinases. Together with the slower synthesis rate (Seene *et al.*, 2004) this may also explain the decrease in the MyHC IIb isoform in FT muscles during six weeks of endurance exercise.

The turnover of MyHC and MyLC isoforms provides a mechanism by which the type and amount of protein can be changed in accordance with the needs of contractile machinery during the adaptation to the exercise training (Seene *et al*., 2004). Our study demonstrates that the turnover rate of MyHC isoforms shows differences between the FT muscles. The turnover rate is faster in FT muscles with a higher oxidative potential. Myosin turnover supports qualitative remodelling of FT muscles, so that the former pattern of MyHC and MyLC isoforms is changing, and the contractile process is better suited to new conditions of long-lasting muscle activity.

As all myofibrillar proteins are in the continuous process of synthesis and degradation, changes in the turnover rate of the main contractile protein myosin molecule characterize these main renewal processes in the contractile apparatus during the adaptation to the aerobic endurance training. Changes in the turnover rate of MyHC isoforms in the FT muscles during the adaptation to the endurance training characterize also changes in the myofibrillar apparatus through protein metabolism. The latitude of changes (increase, decrease) in the turnover rate of a certain myosin isoform shows also the significance of MyHC isoforms in the process of adaptation to the endurance training. The present study also revealed that during endurance training MyHC IIa, IIb, IId, and MyLC 2_s and 3_f isoforms in FT muscles reflected explicitly the process of adaptation through changes in the realtive content of myosin isoforms.

When seeking an answer to the question how does the prolonged mechanical activity affect the contractile apparatus in FT muscles, it is expedient to begin with the backbone – the myosin molecule. Although the exact role of MyLC isoforms in FT muscles during the adaptation to the aerobic endurance training is not fully known, changes in the relative content of MyLC isoforms and their relations with the character of training show that they play an important role in the process of modulation of contractile machinery during the increase in the oxidative capacity and more intensive degradation rate of contractile proteins (Seene *et al*., 2004; Seene *et al*., 1999). Simultaneously with increased degradation of contractile proteins, endurance training also increased the degradation rate of MyHC isoforms. The degradation rate of MyHC isoforms increases in spite of the increase in the oxidative potential of the FT skeletal muscle. The decrease in the expression of MyHC IIb isoform in FT muscles is caused by the intensive degradation of the isoform during endurance training, which is probably the main reason for unchanged turnover rate of MyHC IIb isoform in endurance-trained rats. During the adaptation to the long-lasting endurance exercise a decrease in MyHC IIb isoform in FT skeletal muscle points to the transformation of the muscle contractile apparatus in accordance with the increase in muscle oxidative capacity. This adaptational process shows coordination between changes in oxidative capacity and contractile machinery in skeletal muscle during the adaptation to the endurance training first of all in relation to muscle metabolism. Adaptational processes in FT muscles during

endurance training show a high potential of recruiting these muscles in endurance training.

3. Regeneration of the fastest isoforms of MyLC and MyHC

An excessive increase in the volume of endurance training leads to a decrease in physical working capacity. This is mainly caused by increased degradation and decreased synthesis of MyHC. During this process changes in MyHC composition depend on the twitch characteristics of muscle. In the Sol muscle, where the MyHC I isoform predominates, and in the Pla muscle, where the MyHC IId isoform predominates, the relative content of these dominating MyHC isoforms increases during an excessive increase in the training volume. At the same time the content of MyHC IIa isoform decreases in the ST muscle and increases in the FT muscle. This may be caused by the different sensitivities of the same MyHC isoforms in ST and FT muscles to the proteinases or by differences in the expression of MyHC or other isoforms of myofibrillar proteins in different muscles (Seene *et al*., 2003).

As regeneration of FT muscles needs more time than that of ST muscles, and the regeneration of contractile proteins in FT muscles is slower, it was proposed that the low oxidative capacity is the reason for that (Seene and Umnova, 1992). As noted, the fastest MyHC IIb isoform and MyLC 3_f isoform in FT muscle fibres have a high affinity for coexistence. It has theoretical as well clinical importance to understand regeneration of these isoforms. Nerve-intact auto-grafting of the EDL muscle revealed no significant difference between the mass of control and grafted muscles. It proved that the chosen way to study the process of regeneration of myosin fastest isoforms in autografted muscles was right.

In autografted EDL muscle the MyHC IIb isoform decreased in comparison with the control group. It may be caused by temporal loss of functional innervation, even for a short time, which causes permanent changes toward the slower type of MyHC isoform pattern among fast MyHC isoforms in autografted FT muscles. In contrast to the MyHC isoforms, MyLC isoforms revealed a difference between the denervated and innervated grafts during the first ten days, but later the content of MyLC isoforms in the grafts became similar. It has been shown that in the denervated Sol muscle, the composition of MyLC isoforms is similar to the non-denervated EDL muscle (Carraro *et al.*, 1983). Thus, the expression of fastest MyLC isoforms is independent of innervation. The changes in MyLC content in grafted muscles are probably not caused by the difference in the expression in the mRNA level since the expression of fast MyLC mRNAs does not depend on the innervation of muscle (Esser *et al.*, 1993).

CONCLUSIONS

1. There is a ten-time difference in the relative content of MyLC slow and fast isoforms between fast-twitch and slow-twitch skeletal muscles. The differences between fast-twitch muscles does not exceed 1.4 times.

   The differences in the MyHC pattern between two fast-twitch muscles are considerably bigger than these of MyLC isoforms. Up to two times difference exists in the relative content of MyHC fastest isoform between these muscles.
2. Endurance training increases the MyHC degradation rate whereas the MyHC I and IIb isoforms are more sensitive to the degradation than MyHC IIa and IId isoforms in fast-twitch muscles. The MyHC isoforms, except for IIb isoform, turn over faster in endurance-trained fast-twitch muscles.

   The turnover rate of MyLC isoforms does not change significantly. The ratio of MyLC 3_{fast} isoform to the regulatory LCs and the ratio to the alkali MyLC fast isoforms increase in endurance-trained skeletal muscles.
3. An excessive increase in the training volume leads to exhaustion, increases the relative content in the MyHC I isoform is the slow-twitch muscle but decreases it in the fast-twitch muscle. Also an excessive increase in the training volume decreases MyHC IIb isoforms in fast-twitch muscles.

   Endurance training increased the relative content of MyLC 3_{fast} isoforms in fast-twitch muscles; exhaustive exercise decreased it.
4. Regeneration of MyHC after exhaustive exercise takes at least 24 hours, whereas regeneration of MyLC regimes twice less. Regeneration of MyHC IIb and MyLC 3_{fast} isoforms proceeds at different speeds in the fast-twitch muscle after grafting. Regeneration of MyHC IIb isoform takes more than sixty days; MyLC 3_{fast} isoform regenerates during thirty days after muscle damage.

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SUMMARY IN ESTONIAN

Erinevused müosiini kergete ja raskete ahelate isovormilises kompositioonis kiiretes ja aeglastes skeletilihastes – vastuvuspidavustreeningu mõju

Töös on uuritud müosiini kergete ja raskete ahelate isovormilist kompositiooni kiiretes ja aeglastes skeletilihastes, treeningu mõju eelnimetatud isovormide ekspressioonile, uuenemis- ja degradatsiooni kiirusele.

Skeletilihastes erineb müosiini kergete ahelate kiirete isovormide suhteline sisaldus kümme korda, kiirete lihaste vahel ei ületa see erinevus 1,4 korda. Aeglastes ja kiiretes skeletilihastes müosiini raskete ahelate kiirete isovormide suhteline sisaldus oluliselt erineb.

Vastupidavustreening intensiivistab müosiini rastete ahelate degradatsiooni, kusjuures müosiini raskete ahelate I ja IIb isovorm on degradatsioonile tundlikumad kui IIa ja IId isovorm. Vastupidavustreeningu mõjul müosiini raskete ahelate isovormid uuenevad treenitud loomadel kiiremini kui treenimata loomadel, väljaarvatud müosiini raske ahela IIb isovorm. Samal ajal müosiini kergete ahelate uuenemiskiirus oluliselt ei muutu.

Kurnavad koormused kutsuvad esile müosiini raskete ahelate I tüüpi isovormi suhtelise sisalduse suurenemise aeglastes lihastes, kiiretes lihastes aga aeglase isovormi ja kõige kiirema isovormi- IIb suhtelise sisalduse languse.

Kiiretes skeletilihastes põhjustab vastupidavustreening müosiini kergete ahelate 3_f isovormi suhtelise sisalduse suurenemise, kurnavad koormused aga languse.

Müosiini kergete ahelate 3_f isoformi ning aluseliste ja regulatoorsete isovormide suhe suureneb vastupidavustreeningu mõjul.

Müosiini raskete ja kergete ahelate suhteline sisaldus taastub erineva kiirusega. Müosiini raskete ahelate taastumiseks kurnava treeningu järgselt kulub rohkem kui 24 tundi, kerged ahelad taastuvad oluliselt kiiremini. Müosiini kergete ahelate 3_f isovormi ja müosiini raskete ahelate IIb isovormi taastumine pärast kiire lihase siirdamist toimub samuti erineva kiirusega. Müosiini raskete ahelate IIb isovorm vajab selleks enam kui 60 päeva, kergete ahelate 3_f isovorm aga 30 päeva.

ACKNOWLEDGEMENTS

This study was carried out in Chair of Functional Morphology, Institute of Exercise Biology and Physiotheraphy Faculty of Exercise and Sport Sciences at the University of Tartu.

The work was suppoted by grant No 3266, 4611 of the Estonian Science Fondation and by the Estonian Ministry of Education and Research.

My sincerest thanks and respect belong to:

- My supervisor professor Teet Seene for enthusiasm, continuous support, and guidance, which made this dissertation possible;
- My colleagues, dr. Priit Kaasik, Raivo Puhke, dr. Ando Pehme in the Chair of Functional Morphology for their warm attitude, good collaboration, fruitful discussions and support;
- Mrs Piret Pärsim for help in the preparation of the manuscript and kind support;
- My former colleague and coauthor Jaanus Järva;
- All my coauthors for their excellent contributions;
- All my colleagues in the Institute of Exercise Biology and Physiotheraphy for their support friendly atmosphere and help;
- Professor Atko-Meeme Viru and Professor Enn Seppet for reviewing my manuscript, for the critical and helpful comments and suggestions in improving this thesis;
- Associated professor Anne-Mai Parring for consultations in statistical analysis;
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1964–1967	Otepää Keskkool
1967–1972	Tartu Ülikool füüsika-keemiateaduskond
1995	Sporditeaduste magister, dissertatsiooni teema: Kehalise aktiivsuse mõju müosiini raskete ahelate isovormide kompositsioonile ja müosiini raskete ahelate uuenemisele
2000–2005	Doktoriõppes (eksternina) Tartu Ülikooli kehakultuuriteaduskonnas

Erialane teenistuskäik

1972–1976	TÜ, arstiteaduskond, spordimeditsiini kateeder, nooremteadur
1976–1986	TÜ, kehakultuuriteaduskond, lihastalituse hormonaalregulatsiooni labor, nooremteadur
1986–1992	TÜ, kehakultuuriteaduskond, lihastalituse hormonaalregulatsiooni labor, teadur
1992–kuni käesoleva ajani	TÜ kehakultuuriteaduskond, spordibioloogia ja füsioteraapia instituut funktsionaalse morfoloogia õppetool, teadur

Erialane enesetäiendus

1992	Kursus: Laboratoorsed katseloomad teadusuuringutes — täna ja homme Djuranäset, Rootsi
1994	Täiendõpe Konstanzi Ülikoolis

Erialaroganisatsioonid

Eesti Füsioloogia Seltsi liige
Eesti Olümpiaakadeemia asutajaliige

Teadustegevus

Peamised uurimisvaldkonnad: Skeletilihase kontraktiilsed ja regulatoorsed valgud

Kokku on ilmunud 140 teaduspublikatsiooni, sh 21 artiklit rahvusvahelistes eelrefereeritavates ajakirjades ja kogumikes.