DISSERTATIONES KINESIOLOGIAE UNIVERSITATIS TARTUENSIS
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COMPOSITION AND TURNOVER OF MYOFIBRILLAR PROTEINS IN VOLUME — OVERTRAINED AND GLUCOCORTICOID CAUSED MYOPATHIC SKELETAL MUSCLE

PRIIT KAASIK

TARTU UNIVERSITY
PRESS

Department of Functional Morphology, Institute of Exercise Biology and Physioterapy, University of Tartu, Tartu, Estonia

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Opponents: Professor Jørgen Jensen, National Institute of Occupational Health, Oslo, Norway
Professor emer. Atko-Meeme Viru, Institute of Exercise Biology and Physiotherapy, University of Tartu, Estonia

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1. **Seene T., Kaasik P., Alev K., Pehme A., Riso E. M.** Composition and turnover of contractile proteins in volume — overtrained skeletal muscle. Int. J. of Sports Medicine. 2004, 25: 1–8.

2. **Seene T., Kaasik P., Pehme A., Alev K., Riso E. M.** The effect of glucocorticoids on the myosin heavy chain isoforms' turnover in skeletal muscle. Journal of Steroid Biochemistry & Molecular Biology, 2003, 86, 2, 201–206.

3. **Kaasik P., Seene T., Umnova M., Alev K**. The mechanism of action of glucocorticoids in the rat skeletal muscle. Baltic J. Lab. Anim Sci, 2000, 10, 185–193.

4. **Seene T., Umnova M., Kaasik P.** The Exercise Myopathy. Overload, Performance Incompetence, and Regeneration in Sport. Ed. by M. Lehmann (*et al*.) Kluwer Academic Plenum Publishers, NY, 1999, 119–130.

5. **Kaasik P., Alev K., Seene T.** The effect of activity on the rat skeletal muscle contractile apparatus. Scand. J. Lab. Anim. SCI. 1996, 23, 1, 35–39.

ABBREVIATIONS

EDL	*extensor digitorum longus* muscle
F-G	fast-glycolytic muscle fibres
F-OG	fast-oxidative-glycolytic muscle fibres
FT	fast-twitch
G	glycolytic muscle fibres
HSP	heat shock proteins
IGF-I	insulin like growth factor-I
MGF	mechano growth factor
MyHC	myosin heavy chain isoform
MyLC	myosin light chain isoform
O	oxidative muscle fibres
OG	oxidative-glycolytic muscle fibres
Pla	*plantaris* muscle
PWC	physical working capacity
ROS	reactive oxygen species
SDS-PAGE	sodium dodecylsulphate polyacrylamide gel electrophoresis
S-O	slow-oxidative muscle fibres
Sol	*soleus* muscle
ST	slow-twitch
3-MeHis	3-Methylhistidine

INTRODUCTION

The physiological, metabolic and biochemical factors that may limit exercise performance during different exercise conditions are developing in the specific training process (Noakes, 2000). Endurance training results in increased mitochondrial density, capillary supply, changes in key metabolic enzymes, increased maximal oxygen uptake (Holloszy & Booth, 1976) and 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).

The nature of the physiological adaptation that occurs in response to endurance training has been extensively studied. In contrast, fewer studies have evaluated the role of training volume and recovery state between exercise sessions on the individual myofibrillar proteins' turnover rate and the accompanying changes in the myosin heavy and light chain isoforms' pattern in slow- and fast-twitch muscles (Seene & Alev, 1991).

From the scientific standpoint, exercise training does not solely consist in repetitive physical exercises, but also encompasses regular regeneration as an integral part of a successful training programme (Foster *et al*, 1995; Steinacker *et al*, 1999). Systematic recovery periods in the training process are necessary to prevent the overtraining syndrome and to obtain overreaching for further performance improvement (Lehmann *et al*, 1993). If the time to the next training session is too long, the overcompensation will regress to the original functional state and progressive improvement will not occur. Conversely, if the training stimulus is provided too frequently, so that it interrupts the recovery phase, adaptation will not occur. Overloading is a natural part of a modern endurance athlete's training process which provides stimuli for adaptation and supercompensation. In accordance with the latter statement, the training stimulus must be strong enough to induce disturbance of homeostasis so that the body has to initiate reactions to adapt to the training stimulus. As repetitive overloading and lack of recovery are leading to the overreaching and overtraining syndrome, consequently, the threat of overtraining is a major problem among competitive athletes. Hooper *et al* (1995) suggested that the volume of training, rather than intensity, may be that major contributing factor in the development of overtraining syndrome. If the overload has been properly designed, a progressive improvement in performance will result (O'Toole, 1998). The main question is how much rest and recovery is necessary in a training program. It is well known that many top level endurance athletes are training with high volume without performance improvement. Consequently, the main problem of athletes is to find optimal training volume. Establishing the optimal training regimen is complicated as both the volume of training and recovery period that is optimal for performance improvement in endurance training is difficult to determine: what is optional for some athletes, may undertrain or overtrain others. Furthermore, in modern training, overloading is a natural part of an

athlete's training process that provides stimuli for adaptation and supercompensation.

The most typical ultrastructural changes occur in endurance training in slow-twitch (ST) oxidative (O) and fast-twitch (FT) oxidative-glycolytic (OG) muscle fibres. The lesions in myosin and actin filaments and distributed regularity of Z-disc in sarcomeres, destruction of mitochondria, dilation of the terminal cisternae of the sarcoplasmic reticulum and the tubules of T-system are examples of these changes. During endurance training ultrastructural changes in FT glycolytic (G) muscle fibres are considerably less evident than in OG fibres.

The overtraining syndrome is accompanied with serious ultrastructural changes in skeletal muscle. Due to the destruction of myofibrils and, mainly, atrophy of OG fibres, exercise myopathy may be accompanied with the overtraining syndrome (Seene *et al*, 1995; Lehmann *et al*, 1999).

Similar functional and structural changes in skeletal muscle in glucocorticoid and exercise myopathies have given ground for speculations that exercise myopathy might be a mild form of corticosteroid myopathy (Lehmann *et al*, 1999).

Administration of large doses of glucocorticoids induces muscle myopathy. The ultrastructural study showed the disarray of thick myofilaments in FT G fibres in dexamethasone-treated rats. The destructive process of myofilaments begins from the periphery of myofibrils, spreads to the central part of sarcomere near the H-zone and is distributed all over the A-band (Seene *et al*, 1988).

The thin filaments and Z-line are much more resistant to any catabolic action of dexamethazone than thick filaments in glycolytic muscle fibres. The myofibrils of glycolytic muscle fibres are thinner in dexamethazone-treated rats. This is caused by the splitting of myofibrils. In OG fibres the destruction of thick myofilaments was remarkably less pronounced. Myofibrils were structurally normal in the dexamethazone-treated *soleus* (Sol) muscle. There was evidence of increased lysosomal activity in the G muscle fibres and in the satellite cells of dexamethazone-treated rats (Hussar *et al*, 1992).

Although there are some similarities between the above mentioned two myopathies (decrease of muscle grip strength, and physical working capacity, decreased contractile proteins synthesis rate, increased degradation rate), the main destructive changes occur in different types of FT muscle fibres (Seene, 1994; Seene *et al,* 1995). However, the differences in the myosin heavy chain synthesis, degradation and turnover rate on the different muscle and muscle fibre level are still open.

Protein isoforms of the contractile apparatus are interchangeable and a very large number of phenotypes can be generated by their combinations. There are only a few stable phenotypes which correspond to the known main four muscle fibre types and have properties that depend on their total protein content. Among those, myosin isoforms are the major determinants of the large functional heterogenity of skeletal muscles (Pette, 2001). In skeletal muscle fibres myosin works within the structure of the sarcomere. Contractile properties of the muscle fibres depend on the myosin heavy chain (MyHC) isoform com-

position but at the same time they may be regulated by the presence of the isoforms of other myofibrillar proteins like regulatory and minor proteins (Pette & Staron, 2000). Muscle protein content and quality depend on the integrity of the remodeling process of muscle during endurance training. The remodeling involves removal of an old protein and replacement with new (Booth *et al*, 1998).

It has been shown that ST muscles are more resistant to high volume endurance exercise, leading to the overtraining syndrome, than FT muscles, and this may be related with MyHC composition. Unfortunately, we do not know how the same MyHC isoforms are changing in ST and FT muscles during the increase of training volume, and whether MyHC isoforms in different muscles possess a different sensibility in dependence of the oxidative potential of muscles or not.

REVIEW OF LITERATURE

1. THE EFFECT OF TRAINING VOLUME ON SKELETAL MUSCLE

Despite the elevation in mechanical activity, endurance exercise does not result in hypertrophy of the muscle groups involved in the exercise response because the level of force production is relatively small compared with their maximal force-generating capacity. However, it has been reported that there may be subtle enlargement of those fibres comprising ST motor units heavily utilized during each training session (Smerdu *et al*, 1994; Freyssenet *et al*, 1996). During endurance training transformations in the expression of MyHC IIa and IIx isoforms with more economical cross-bridge cycling kinetics are expressed instead of fast MyHC IIb isoforms (Baldwin & Haddad, 2002). Consequently, when exercise of a given submaximal intensity and duration is performed in the trained state, the exercise usually becomes more economical for the individual to perform.

High volume training protocols for both top athletes and animal models lead to chronic fatigue and decreased exercise performance and are characterized by changes in skeletal muscle, such as irregular fibre size and distribution and an alteration in mitochondria structure with lipid inclusions (Lambert *et al*, 1999). Morphological, as well as biochemical findings in previous research have shown that during exhaustive endurance training which leads to the overtraining syndrome, changes in the skeletal muscle myofibrillar apparatus, particularly destruction of contractile proteins, are the most significant factors in the process of decreasing exercise performance (Seene *et al*, 1995; Lehmann *et al*, 1998). Although widely studied, the ultimate causes and pathophysiological nature of the overtraining syndrome are not fully understood (Foster *et al*, 1999b), particularly on the level of skeletal muscle (Lehmann *et al,* 1998).

1.1. The Effect of Endurance Training on the MyHC Isoforms in Different Types of Skeletal Muscle Fibres

Skeletal muscle is a plastic tissue capable of modifying its contractile and metabolic properties with increased use. Training and stimulation studies have demonstrated significant changes in the relative expression of fast and slow MyHC isoforms, most commonly a shift from the subgroup of fast isoforms to slower oxidative isoforms (MyHC IIa and I). The importance of contractile activity in determining the muscle phenotype is widely recognized. An important role in modulating phenotype has been assigned to the motoneuron. It has been demonstrated that the extent and modality of neuronal activity is crucial for regulating contractile properties such as speed, strength and endurance.

World-class marathon runners and ultra-endurance athletes have been reported to possess remarkably high type I fibre numbers in their trained muscle groups (Andersen *et al*, 2000), whereas muscles of sprinters and weightlifters predominantly consist of IIA/IIX fibres (Andersen *et al*, 2000). It further appears that extreme usage induced alterations of the muscle MyHC isoform profile result in hybrid fibres that express a combination of MyHC isoforms such as type I/IIa. To which extent these patterns of MyHC gene expression are conditioned by genetic predisposition or by the specificity of training, is unresolved (Baldwin & Haddad, 2001).

The effect of endurance exercise on the MyHC profile appears to be both muscle-specific and dose-dependent. For example, when rodents are trained to run at moderate to high intensity for several weeks, the running effect on the MyHC profile of the Sol are manifested only when animals run for longer durations (Demirel *et al*, 1999). In fast muscles, which have a composition bias to type IIx and IIa MyHC expression, both the type IIa and IIx MyHC are up-regulated relative to the sedentary state, whereas the IIb MyHC is significantly down-regulated under these training conditions (Demirel *et al*, 1999). If running is extended for longer durations, it is possible to induce increased expression of the type I MyHC in fast muscles (Demirel *et al*, 1999).

1.2. The Effect of Endurance Training on the Myofibrillar Proteins in Different Fibre Types

It has been shown that during endurance training type II fibres transform into type I fibres not only on the level of ultrastructural and metabolic function, but also on the molecular structure level of contractile proteins (Thayer *et al,* 2000). It is still unclear how changes in the contraction speed, one of the functional factors known to change following endurance training, are caused and on which stage they occur.

Protein isoforms of the contractile apparatus are interchangeable and a very large number of phenotypes can be generated by their combinations. There are only a few stable phenotypes which correspond to the known main four muscle fibre types (type 1, 2A, 2D, 2B) and have properties that depend on their total protein content (Pette & Staron, 2000). Among those, myosin isoforms are the major determinants of the large functional heterogenity of skeletal muscles. In skeletal muscle fibres myosin works within the structure of the sarcomere. Contractile properties of the muscle fibres depend on the MyHC isoform composition but they may be simultaneusly regulated by the presence of the isoforms of the other myofibrillar proteins (Bottinelli, 2001). Muscle protein content and quality depend on the integrity of the remodeling process of muscle during endurance training. The remodeling involves removal of an old protein and replacement with new (Booth *et al*, 1998).

It has been shown that ST muscles are more resistant to high volume endurance exercise, leading to the overtraining syndrome than FT ones, and the reason of that may be related with the MyHC composition. The multidirectional changes of the same MyHC isoforms in ST and FT muscles during the increase of training volume not only support the different resistance of ST and FT muscles, but also show the different sensibility of the same MyHC isoforms in different muscles (Pette, 2001). C-protein seems to play the essential role in correct thick filament formation during myofibrillogenesis, modulating muscle contraction, and increasing the maximum shortening velocity (Hofmann *et al*, 1991). C-protein can either bind actin and myosin or affect the mechanical properties of myosin cross-bridges by linking the S2 segment of myosin to the backbone of the thick filament (Hofmann *et al,* 1991). The Z-disc protein α-actinin and regulatory proteins Tn-I and TM seem to be more resistant to excessive training volume. Regulatory protein Tn-T, like minor C-protein is very sensible to high training volume and together with MyHC isoforms may play the key role in the changes of functional properties of contractile machinery during the period of excessive training volume.

2. THE EFFECT OF ENDURANCE TRAINING ON THE SYNTHESIS RATE OF MYOFIBRILLAR PROTEINS

Changes in the type of protein and the time course of amino acid incorporation following exercise are related to the metabolic characteristics of the muscle fibres and intensity of the exercise (Baldwin & Haddad, 2002). During endurance exercise, most of the skeletal muscle protein synthesis is suppressed and protein breakdown is increased (Baldwin & Haddad, 2002). During recovery after exercise, the synthesis rate of contractile proteins is increased, but this process depends on the character of exercise and on the metabolic type of skeletal muscle fibres (Seene & Alev, 1991). It has been shown that in endurance trained rats the turnover rate of the two main muscle contractile proteins, actin and myosin, proceeds faster, an increased number of satellite cells under the basal membrane of skeletal muscle fibres has been observed (Seene & Umnova, 1992). Hence the faster turnover rate of MyHC and actin is accompanied by hyperplasia of skeletal muscle in endurance-trained rats. Thus, changes in the turnover rate of muscle contractile proteins during exercise and recovery period after exercise reflect the functional conditions of the contractile apparatus in different types of skeletal muscle fibres and have a physiological significance (Lehmann *et al*, 1999). The synthesis rate of actin, tropomyosin and myosin light chain (MyLC) are less sensitive to the volume of endurance training than MyHC (Seene *et al*, 1995).

Unfortunately, there are at present no concrete data available in literature about the effect of endurance training volume dependent changes in the synthesis rate of individual myofibrillar proteins.

3. THE EFFECT OF ENDURANCE TRAINING ON THE DEGRADATION RATE OF MYOFIBRILLAR PROTEINS

Muscle protein degradation is transiently elevated during muscle atrophy, enhancing the transition to a new steady-state of reduced muscle mass (Baldwin & Haddad, 2002). In the new steady state, both the synthesis and degradation rates are lower relative to the steady-state maintenance of normal muscle mass (Thomason, 1998). Of the four MyHC isoforms expressed in skeletal muscle, fibres expressing chiefly the type I MyHC isoform seem to be the primary target for protein degradation processes during the states of unloading. In fact, the half-life for the degradation rate of skeletal myosin comprises is less than 7 days, demonstrating that atrophy of slow muscle fibres is a rapidly occurring process (Baldwin & Haddad, 2002).

On the other hand, it has been shown that the destruction of thin and thick myofilaments in trained rats is mostly located in the peripheral myofibrils of FT OG muscle fibres (Seene & Umnova, 1992). In the sarcoplasm of these muscle fibres there occurred secondary lysosomes, autophogic, vacuoles, containing degenerating mitochondria and membranous remnants. In some axon terminals lysosome-like bodies, autophagosomes and extensive membrane structures could be found (Seene & Umnova, 1992).

Although the precise pathways of protein degradation are poorly understood, recent studies implicate the involvement of the adenosine triphosphate-dependent ubiquitin/proteosome pathway in the degradation of contractile proteins (Solomon *et al*, 1998). Consequently, there is the need for focusing research on this system to understand the involvement of the mentioned pathway as part of the overall protein turnover mechanism or mechanisms under a variety of physiological states.

4. REASON OF THE OVERTRAINING SYNDROME DEVELOPMENT

It is known that responses to training are highly complex and individual (Foster *et al*, 1999a). In very long distance competitions, such as marathon running, there is the concept of the "collapse point." The "collapse point" hypothesis proposes that the weekly training volume needs to be at least 2.5 times the racing distance (Foster *et al*, 1999a). Progressive overload is the foundation of all successful training. According to the general adaptation syndrome, stress causes a temporary decrease in function followed by an adaptation that improves function. In the training response, overload is the stress that causes fatigue and improved performance is the adaptation (O'Toole, 1998).

High volume endurance exercise disrupts body homeostasis, and the body has to recover. Therefore, two responses must be paid attention to: the musculoskeletal system's response to an appropriate or inappropriate training load, and

the effect this response or adaptation has on injury or the potential of injury. Musculoskeletal overtraining may occur as a result of inappropriate stress. Inappropriate volume or intensity of exercise may cause a maladaptive cellular or tissue response due to an imbalance between load and recovery (Foster *et al*, 1999b). The oertraining syndrome is not completely understood, but accumulating evidence indicates that disruptions in cellular homeostasis appear to be key factors in the process. Tissue effects arise from these cellular disruptions. Overtraining can be defined as stress-recovery imbalance, that is too much stress combined with too little time for regeneration (Lehmann *et al*, 1999). Short-term overtraining can be seen as a usual part of athletic training which, also called overreaching or supercompensation training, leads to a state of overreaching in affected athletes (Lehmann *et al*, 1999). This state of overreaching is characterized by a transient performance incompetence which is reversible within a short-term recovery period and can be rewarded by a state of supercompensation (Foster *et al*, 1999a; Lehmann *et al*, 1999).

4.1. The Role of Reactive Oxygen Species in the Development of the Overtraining Syndrome

Reactive oxygen species (ROS) are involved in the tissue damages (Pansarasa *et al*, 2002). The reactive species include superoxide anion, hydrogen peroxide, and hydroxyl radical. ROS may cause cell injuries such as lipid peroxidation, enzyme inactivation, changes in intracellular redox state and DNA damage (Halliwell & Gutteridge, 1985).

Cells possess enzymatic defense systems to reduce the risk of oxidative injury, i.e. superoxide dismutase, glutathione peroxidase and catalase with superoxide radicals and organic hydrogen peroxides, respectively.

There is evidence that an increase in ROS production occurs during physical exercise and the resulting oxidative damage arises in muscle, liver, blood, and other tissues (Venditti et al, 1997; Itoh *et al,* 1998). Exhaustive physical exercise has been associated with enhancement of oxygen consumption in skeletal muscles (Packer, 1986), increase in lipid peroxidation, and inhibition of key mitochondrial enzymes such as citrate synthase and malate dehydrogenase (Ji *et al*, 1988). In contrast, mild and regular endurance training does not lead to functional damage and promotes muscular adaptation.

5. THE EFFECT OF ENDURANCE TRAINING ON THE CHANGES OF GROWTH FACTORS

Signaling pathways and secondary messenger factors are involved in transcriptional-translational-posttranslational processes. Insulin like growth factor-I (IGF-I) has been implicated as a factor that can affect many steps in the control

of gene expression, including cell proliferation, differentiation, and degradation processes (Adams, 1998). It is widely accepted that many of the anabolic effects of growth hormone may result from a growth hormone-stimulated increase in IGF-I production. IGF-I has been shown to simulate amino acid transportation, which is essential to tissue growth. Recent studies suggest that autocrine/ paracrine processes involving muscle-derived IGF-I may play a pivotal role in linking the mechanical stimulus to the muscle's morphological and bio-mechanical adaptations (Goldspink, 2000). Goldspink has discovered that in response to stretch or increased mechanical activity, the muscle produces locally a special isoform of IGF-I (autocrine) that is directly linked to the activation of gene expression necessary for muscle repair, maintenance, and remodeling. The product of this isoform is called the mechano growth factor (MGF) to differentiate it from the liver IGFs that have more systemic action (Goldspink, 1999). There are no exact data suggesting the role of IGFs in dependance of the volume of endurance training.

6. THE EFFECT OF ENDURANCE TRAINING ON THE CHANGES OF HEAT SHOCK PROTEINS

It has been shown that heat shock proteins (HSP) play an important role as intracellular chaperones in the immune system and may protect cells from the harmful effects of enviromental stress factors (Locke & Nobl, 1995; Fehrenbach *et al*, 1999). Besides their protective effects, HSPs play a functional role in antigen processing and presentation by major histocompatibility complex I MyHC I (Binder *et al*, 2001). It has recently been discussed that HSPs may also function as extracellular signals to activate the immune response (Moseley, 2000).

Furthermore, it has been shown that endurance exercise is a powerful stimulus of intracellular HSP expression in immune cells, in muscle and other tissues like myocardium, liver, spleen, and brain (Locke *et al*, 1990; Liu *et al*, 1999; Fehrenbach *et al*, 2000). An increased expression of heme oxygenase-1 in leucocytes, which appears only after long, intensive competitive endurance exercise, is indicative that the duration of endurance exercise plays an important role in the activation of the anti-stress system. The release from intact muscle cells may be excluded because the increase of HSP72 in the peripheral blood preceded any HSP72 increase in exercising muscle (Walsh *et al*, 2001), and non-damaged muscle did not release HSP72 into the circulation (Fabbraio *et al*, 2002). On the other hand, HSP72 increased in muscle and early damage of skeletal muscle cells has been described after intense endurance exercise followed by secondary immunological changes (Tidball, 1995). Necrotic cells released HSP72, delivered a maturation signal to dendritic cells and activated the NF-kappaB-pathway (Basu *et al*, 2000).

It has been shown that prolonged, competitive endurance exercise induces a more pronounced response of extracellular HSP72 in the peripheral blood of endurance athletes compared with more intensive but shorter exercise (Fehrenbach *et al*, 2002).

7. ARE THERE ANY SIMILARITIES BETWEEN THE OVERTRAINING SYNDROME AND CORTICOSTEROID MYOPATHY?

The catabolic effect of glucocorticoids is realised on sarcolemma and on skeletal muscle contractile proteins (Lehmann *et al*, 1999). Besides decreased incorporations of amino acid into MyHC (Seene *et al*, 1994), there is some evidence of a depressed turnover of MyHC towards glycolytic type II to oxidative type II to oxidative type I fibres (Seene & Alev, 1991). On the one hand, similar excess plasma glucocorticoid concentrations can be observed in Cushing's syndrome and in healthy athletes during prolonged exhaustive exercise (Lehmann *et al*, 1999).

According to Lehmann *et al* (1999) mechanisms underlying an overtraining-related performance incompetence can be described in affected athletes as overtraining myopathy, mild form of sympathetic nervous system insufficiency, mild form of adrenocortical insufficiency and mild form of hypothalamo — pituitary insufficiency in an advanced stage. These findings negatively influence maximum muscle power, maximum energetic competence, and drive, thus explaining performance incompetence in affected athletes as knewn in myopathic patients (Lehmann *et al*, 1999).

As far is known at present, overtraining myopathy is characterized by depressed turnover of MyHC in FT muscle fibres (Seene & Alev, 1991), depressed neuromuscular and depressed alpha-motoneuron excitability (Lehmann *et al*, 1999). Unfortunately, we do not know at present whether some existing similarities in lesions of myofibrillar apparatus in case of the overtraining syndrome and corticosteroid myopathy give sufficient reason to talk about the same mechanism of development of two myopathies of different origin.

8. MECHANISMS OF DEVELOPMENT OF CORTICOSTEROID MYOPATHY

A number of mechanisms have been proposed to explain the action of glucocorticoids on skeletal muscle. As glucocorticoid treatment alters muscle protein and carbohydrate metabolism (Golberg *et al,* 1980), it has been suggested in most studies that the main reason for muscle weakness and wasting may result from altered muscle metabolism with net protein catabolism (Koski *et al*, 1974). It has also been shown that glucocorticoids can reduce mitochondrial respiration

and the excitability of skeletal muscle sarcolemma, the suggestion being that the difference in response to streroid treatment may reflect structural and functional differences in fast and slow muscle membrane systems (Seene, 1994).

Later it has been demonstrated that hormone treatment did not produce muscle weakness by impairing sarcolemma excitability or excitation-contraction coupling, but the weakness resulted from muscle atrophy (Thomas, 1982). As a decrease in the in *vivo* resting membrane potential of muscle fibres was recorded only in *extensor digitorum longus* (EDL) muscle but not in other muscles composed predominantly of FT fibres, and as glucocorticoid treatment did not produce any alteration in the *vivo* measured resting potentials or excitability of EDL muscle fibres, this depolarisation did not appear functionally significant (Seene, 1994). Iatrogenic steroid myopathy as well as Cushing's disease suggest a selective atrophy of FT muscle fibres. It has been demonstrated that corticosteroid myopathy is accompanied by changes in the muscel protein metabolism, particularly in myofibrillar proteins turnover (Seene & Alev, 1985).

Muscle weakness in case of glucocorticoid myopathy is most probably caused by lesions of the myofibrillar apparatus in muscle fibres and by changes in the state of the neuromuscular synapses (Hickson *et al*, 1984).

However, it is unclear how the catabolic effect of glucocorticoids are realised in the skeletal muscle, particularly selective degradation of intracellular proteins.

8.1. Changes in MyHC Isoform Turnover Rate in Corticosteroid Myopathy

It is well known that iatrogenic steroid myopathy as well as Cushing's disease leads to a marked reduction in muscle mass, wasting of muscle and loss of strength. It has been shown that glucocorticoids increase the expression of myostatin, a negative regulator of skeletal muscle growth in polynucleated muscle fibres, expressing MyHC fast isoforms (Artaza *et al*, 2002). In glucocorticoid myopathic rats the turnover rate of MyHC was lower in the FT muscle fibres, MyLC turned over more rapidly in all types of muscle fibres (Seene, 1994). The turnover rate of MyHC in myopathic rats is related to the changes in MyHC isoform pattern. Unfortunately, we do not know how in myopathic glycolytic G muscle fibres of rats the relative content of MyHC IIb isoform decreases and MyHC IId changes. The ultrastructural have studies have showen the disarray of thick myofilaments in G muscle fibres of glucocorticoid treated rats and increased lysosomal activity in these fibres and in the satellite cells (Seene *et al*, 1988). There were only slight morphological changes in OG fibres and no structural changes in ST O muscle fibres in glucocorticoid treated rats (Seene *et al*, 1988).

Although widely studied, the ultimate causes and pathophysiological nature of changes in MyHC isoforms, their synthesis and degradation rate in myopathic muscle are still unknown. However, this information is necessary for the characterization of changes in kinetic criteria of glucocorticoid myopathic muscle.

8.2. The Role of Alkaline Proteinases in Corticosteroid Myopathy

Alkaline proteinases are synthesised in mast cells. After degranulation the enzyme enters the muscle cell, but the mechanism is unknown. Upon administration of large doses of glucocorticoids there is an increase in the number of mast cells in the perivascular poreous connective tissue of the muscle fibres (Seene, 1994). Around the G muscle fibres, degranulation of mast cells is very clearly expressed (Hussar *et al*, 1992). Simultaneously the number of mast cells in the lymph nodes medulla is considerably decreased. The lymph nodes are probably the sources of the muscle mast cells. This may imply that the increased number of mast cells may be the result of their migration from the lymph nodes. 48 h after glucocorticoid administration the mast cell number in lymph nodes is returned to the control level (Seene, 1994). In the atrophying muscle, myofibrillar destruction starts from those myosin filaments which are located in the peripheral part of the myofibrils (Hickson & Davis, 1981). Thick filaments separate from the adjacent ones, bend and are obviously lysed (Hussar *et al*, 1992). The actin filaments and Z-line seem to be more resistant to the action of alkaline proteinase, in comparison with the myosin filament (Hickson & Davis, 1981).

9. UNSOLVED PROBLEMS

There are many examples of top athletes training with high volume without performance improvement (Foster *et al*, 1999a). The reason is, at least partly in the training program design of modern athletes, using overloading as the natural part of the training process that provides stimuli for adaptation (Foster *et al*, 1999b). Repetitive overloading and lack of recovery leading to the overreaching and overtraining syndrome, seems to be a major problem among competitive athletes. The overtraining syndrome accompanied with serious morphological changes in skeletal muscle, particularly the destruction of myofibrils leads to exercise myopathy (Lehmann *et al*, 1999). It is not fully understood how the ultrastructural as well biochemical and molecular changes in myofibrillar apparatus relate to the functional changes in skeletal muscle during overtraining.

The changes in the expression of individual myofibrillar proteins and their isoforms in volume-overtrained animals may explain the changes in contractile machinery in different types of muscle fibres during volume overload.

The mechanisms underlying volume-caused performance incompetence have been found similar to the corticosteroid caused myopathy (Lehmann *et al*, 1999). Both types of myopathies have been found to be characterized by depressed turnover rate of MyHC in skeletal muscle and depressed neuromuscular excitability (Lehmann *et al*, 1999). Taking into account the above mentioned and some other similarities in lesions of myofibrillar apparatus in the overtraining syndrome and corticosteroid myopathy do not give the ground to say whether these two types of myopathies are of the same or different origin.

AIMS OF THE STUDY

The purpose of the study was to compare the reflection of destruction of myofibrils on the ultrastructural level and changes in myofibrillar proteins in slow-twitch and fast-twitch skeletal muscle and their fibre types in case of volume-overtrained and glucocorticoid caused myopathy.

In detail, the aims were:

1. To compare the destruction of myofibrils and degradation of contractile proteins in skeletal musle in case of volume-overtrained and glucocorticoid caused myopathy.
2. To study changes in the MyHC isoforms synthesis rate and their relative content in slow-twitch and fast-twitch skeletal muscles and their fibre types in volume-ovetrained and glucocorticoid caused myopathy. To clarify the sensitivity of different MyHC isoforms to alkaline proteinase.
3. To find relations between endurance training volume and changes in the synthesis rate of individual myofibrillar proteins and physical working capacity of rats.

METHODS

Use of the animals was in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and was controlled by the Committee of Laboratory Animal Science, University of Tartu.

1. Animals

The used animals were 16–18- and 24-week-old (at the beginning on the experiment) male rats of the Wistar strain (National Laboratory Animal Centre, Kuopio, Finland). All animals were housed in identical environmental conditions in polycarbonate cages, at 21ºC, two per cage at 12/12 hrs light/dark period. The animals were maintained on a constant 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.

2. Endurance Training Protocol

Maladaption to exercise training is mostly an imbalance between stimulus and recovery. We used three different volumes of endurance training protocols. To determine the optimal training volume we measured daily, weekly and total work in kJ.

After a brief 5-day acclimatization that consisted of treadmill running for 5–10 min, rats were subjected to run with the speed of 35 m/min. Rats of the endurance training group were running 5 days per week, the training volume was increased moderately during 6 weeks from 10 min to 60 min per day (Fig. 1). 8 rats were taken after 4 weeks and used as 4-week endurance training group for comparison with the 4-week overtraining group. 8 rats continued the training program until 6 weeks. Rats of the 4-week and 6-week overtraining group ran 7 days per week and the training volume was increased faster than in the endurance training group (Fig. 1) and reached to 2 h 20 min per day on the 4th week. Power of work was 1.5–1.95 W. As shown in Fig 1, the difference in training volume per one training session between the endurance training group and the other groups is ~2.6 times. In comparison, the weekly training volume difference is ~3.7 times between the endurance training and the other groups. The difference in training volume per one exercise session is smaller, as according to the first training protocol there were two recovery days per week. These two days gave the rats time for recovery from exercise stress. The difference in training load per session between the endurance trained and overtrained groups was due to differences in duration of the session and not due to the intensity of exercise. Work per training session, per week, and total work

was expressed in kJ, as well as physical working capacity (PWC) 24 h after the last training session. For the determination of PWC rats were running on the horizontal treadmill with the speed of 35 m/min until exhaustion.

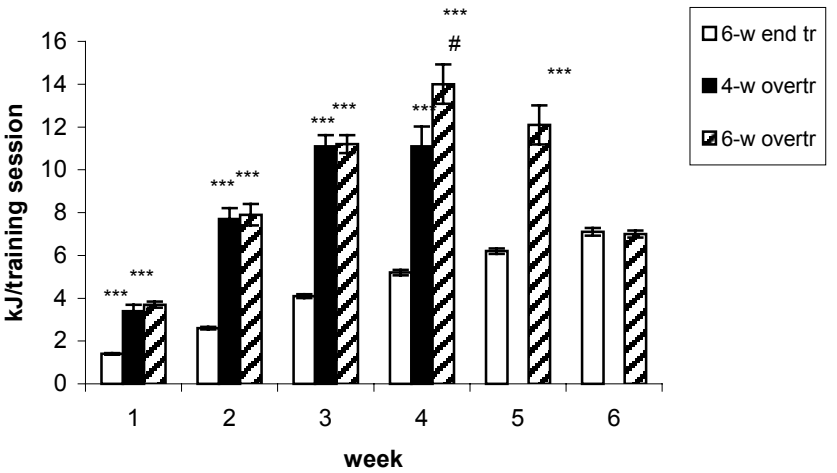

Figure 1. Dynamics of exercise-training volume per daily session
6-w end tr — 6-week endurance training group. Rats of this group had 5 exercise-training days per week (n=8)
4-w overtr — 4-week overtraining group. Rats of this group had 7 exercise-training days per week (n=8)
6-w overtr — 6-week overtraining group. Rats of this group had 7 exercise-training days per week (n=8)
** — $p<0.01$
*** — $p<0.001$
} in comparison with endurance training group
— $p<0.05$ in comparison with 4-week overtraining group

The total work (A) done by animals and the power of exercise (N) was calculated by the following formulas:

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

m – body weight (kg),
V – running speed (m/min);
t – running time (sec);
S – running distance (m).

3. Dexamethasone Treatment

Dexamethasone (Clucocortin-20, Interchemie, Holland) was diluted to 200 μg/ml with 0.15 M NaCl and administered intraperitoneally 100μg/100 g body weight during 10 days. The control animals received appropriate amounts of 0.15 M NaCl.

4. Labelled Amino Acid Infusion and Muscle Removal

Labelled leucine was used for the determination of proteins synthesis rate. Briefly, L- [4.5 – ^{3}H] leucine (170 Ci/mmol) was infused intraperitoneally for 6 h, 250 μCi per 100 g body weight.

In order to investigate the turnover rate of contractile proteins in endurance-trained rats, the double isotope method as described by us previously (Seene & Umnova, 1992) was used. L-[U-^{14}C] Lysine (336 mCi/mmol) 10 μCi per day was discontinued after 5 days and L-[4.5 – ^{3}H] Lysine (40 Ci/mmol) 100 μCi per day was continued for 12 days. The relative turnover rate of the protein fraction was estimated from the ^{3}H/^{14}C ratios. For the same protein turnover rates, the ^{3}H/^{14}C ratios were expected to be the same. Protein with a higher turn-over rate would have a greater ^{3}H/^{14}C ratio.

The animals were anesthetized by intraperitoneal injection of ketamin (Calysol, Gedeon Richter A.O. Budapest, Hungary) and diazepam (Lab Renaudin, France) and sacrificed. The *plantaris* (Pla), EDL and Sol muscles were quickly removed, trimmed clean of visible fat and connective tissue, weighed, frozen and stored in liquid nitrogen pending further processing.

5. Muscle Growth Rate

Fractional muscle growth rate was expressed as the percentage of the growth per day and was calculated in each muscle as the growth rate of protein per day divided by the mean protein mass at the age point and multiplied by 100 to obtain the fractional growth rate in % per day (Garlick *et al,* 1980).

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 $MgCl_2$, 1 mM ditiothreitol, at pH 7.0, and analyzed as total protein fraction. The total muscle homogenate was dissolved in 0.3 M NaOH and was analyzed for radioactivity and protein.

7. Separation of Myofibrillar Protein

Frozen muscles were thawed on ice, cut into small pieces, and washed with five volumes 20 mM NaCl, 5 mM sodium phosphate, 1 mM EGTA (pH 6.5). Myosin was extracted with three volumes 100 mM sodium pyrophosphate, 5 mM EGTA, 1 mM dithiothreitol (pH 8.5); after 30 min of gentle shaking myosin was diluted with one volume glycerol and stored at –20°C (d'Albis *et al*, 1979).

8. Estimation of 3-Methylhistidine (3-MeHis) in Skeletal Muscle and Urine

The 3-MeHis in skeletal muscle and urine was used as an indicator for protein degradation. The determination was performed as described previously (Seene & Alev, 1985). Briefly, 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 achive the concentration of 10–20 mg/ml. 3-MeHis in urine and muscle tissue was estimated with HPLC (Seene & Alev, 1985).

9. Protein: DNA and RNA: protein in skeletal muscle

DNA and RNA contents were determined spectrophotometrically in homogenates as described by Millward and Waterlow (1978).

10. Quantitation of MyHC and Actin

12.5% SDS-PAGE slab gel electrophoresis was carried out according to the procedure of Laemmli (1970). Gels were stained by Coomassie Brilliant Blue R-250 (CBB).

11. Recovery and Hydrolysis MyHC and Actin for Amino Acid Analysis

The content of leucine in myofibrillar proteins was used for expression of synthesis rate per M of leucine. The myofibrillar proteins were electroeluted from 12.5% SDS-PAGE (Laemmli, 1970) according to Hunkapiller *et al* (1983). After staining with Coomassie Brilliant Blue R-250 and detection on 12.5% SDS-PAGE the protein band was sliced and minced with razor blade, and rinsed with water. After soaking the gel in elution buffer (0.1% SDS in 0.05 M TRIS-acetate, pH 7.8) for 5 min and in soaking buffer (2% SDS 0.4 M NH_4HCO_3) for 1.0 h, the electroelution went on in dialyzing bag, using hori-

zontal electrophoresis cell (Gel Electrophoresis Apparatus GNA – 100, Pharmica, Sweden). The running conditions for the elution cell were: power supply 70 V (constant voltage) and current 7 mA for 1.5 h. After elution the samples were collected (1000 μl) and the gel pieces were removed by centrifugation (14,000 g). Residual SDS was removed by the following dialyzing in 1 ml dialysis buffer (0.02% SDS in 0.01 M NH_4HCO_3) and rinsing the samples twice with 1.0 ml deionized water by centrifugation in microconcentration tube (10,000 D) and MyHC and actin were washed from filter with 800 μl deionized water.

Eluent fraction containing myofibrillar proteins (200 μl) was evaporated with nitrogen stream, protein-bound amino acids from 10 μg samples were liberated by hydrolysation at 110°C for 18 h in 200 μl 6 N HCl in nitrogen area, and HCl was evaporated with nitrogen. Leucine quantity in MyHC and actin hydrolysate was determined by using an ultra rapid and sensitive HPLC method for measuring individual free amino acids in biological fluids by Graser *et al* (1985), employing precolumn derivatization with *o*-phtalaldehyde/3-mercaptopropionic acid and using 3-μm-particle-size reversed-phase columns (Hyperchrome, Spherisorb ODS II, 3 μm, 125 × 4.6 with guard columns 10 × 4.6 mm, 5 μm; Leonberg, Germany). Resolution of the amino acid derivatives was accomplished with an acetonitrile gradient in 12.5 mM sodium phosphate buffer, pH 7.2 (Graser *et al,* 1985). The MyHC and actin fraction was analyzed for radioactivity and protein, as well as leucine and expressed as dpm/M leucine/min.

12. Determination of Whole Muscle MyHC Isoform Composition

Aliqots containing 0.5 μg of protein were loaded on the gel after being incubated for 10 min at 65°C in sample buffer containing 62.5 mM Tris-HCl, pH 6.8 20% (vol/vol) glycerol 5% (vol/vol) 2-mercaptoethanol, 2.0% SDS, 0.05% bromphenol blue. MyHC isoforms were separated by 7.2% SDS-PAGE using 0.75 mm thick gel. Electrophoresis lasted for 24 h at 120 V (Hämäläinen & Pette, 1996). Gels were silver-stained by the method of Oakley *et al,* (1980). Protein isoform bands were analysed densitometrically by Image Master® 1 D program, Version 3.0 (Amersham Pharmacia Biotech, UK) and the percentage distribution of the various isoforms was evaluated.

13. Alkaline Proteinase

Alkaline proteinase separation and activity measurement was provided by Dahlmann *et al,* (1981) as described by us earlier (Seene *et al*, 2003). Incubation of myofibrils (5 mg/ml) in vitro conditions with alkaline proteinase

(0.7 u/ml) was provided in 50 mM Tris-HCl, pH 8.5 at 37°C during 20 min, mixed (1/10 v/v) with buffer (62.5 mM, Tris-HCl; pH 6.8, 20% Glycerol, 2% SDS, 5% SDS, 5% β-merkaptoethanol) and incubated 2 min at 100°C.

14. Protein Assay

Total muscle protein and myofibrillar protein were assayed by using the technique described by Bradford (1976).

15. Ultrastructural Studies

Muscle samples for ultrastructural studies were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in graded alcohol and embedded in Epon-812. The ultrathin section was cut from longitudinally and transversely oriented blocks, stained with uranyl acetate and lead hydroxide, using 3-5 blocks from each animal. The number of satellite cells, containing a nucleus, was calculated by electron microscopy per 1000 myonuclei in experimental and control groups. The satellite cell frequency was determined as a ratio of the nucleus-containing satellite cells divided by the total number of myonuclei including the the nuclei of satellite cells (Seene & Umnova, 1992).

16. Statistics

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

RESULTS

1. ENDURANCE TRAINING PROTOCOLS AND PHYSICAL WORKING CAPACITY

In the endurance training protocol the weekly training volume increased during 6 weeks from 7 to 36 kJ and total work was 134 ± 7.1 kJ. The weekly training protocol consisted of 5 exercise days and 2 exercise free recovery days. After 6-week training programme rats had 24 h for recovery and physical working capacity (PWC) was measured. The average PWC in the endurance training group was 31.37 ± 1.56 kJ. The training volume of the 4-week and 6-week overtraining group during the first three weeks was practically the same (Fig. 1). Difference in training volume in the third training week in comparison with the endurance training group was about 3 times. In the 4-week overtraining protocol, the volume of exercise in the last week was kept on the level of the third week in order to compare the effect of different training volumes on the local mechanisms in the skeletal muscle contractile apparatus. During the four weeks of training the volume in the 4-week overtraining protocol increased from 24 to 78 kJ, total work was 235.3 ± 16.2 kJ ($p<0.01$ in comparison with the endurance training group) and the protocol consisted of 7 exercise days per week. The PWC of rats of the 4-week overtraining group was 19.43 ± 1.42 kJ ($p<0.001$ in comparison with the endurance training group). Rats exercising according to the 6-week overtraining protocol had the volume of training increased during the first four weeks from 26 to 97 kJ, then it was decreased during the fifth and the sixth weeks as the rats were not able to keep the appointed training volume (Fig.1). Total work in this group was 390.7 ± 28.1 kJ ($p<0.001$ in comparison with the endurance training group) and the training protocol consisted of 7 exercise days per week. In this group the PWC was 14.32 ± 1.07 kJ ($p<0.001$ in comparison with the 4-week overtraining group).

2. CHANGES IN BODY AND MUSCLE MASS DURING DIFFERENT VOLUMES IS OF ENDURANCE TRAINING

During exhaustive exercise training, peripheral neuromuscular structures are primarily affected. As seen in Table 1, during overtraining muscle mass decreased in all studied muscles as well as muscle fractional growth rate per day (Table 2).

Table 1. Changes in the body weight and muscle weight during endurance and overtraining

Group	Body weight (g)	*Plantaris muscle* (mg)	*Extensor digitarum longus muscle* (mg)	*Soleus muscle* (mg)
Control at the beginning of experiment (n = 10)	355.6 ± 6	331.0 ± 8.1	164.4 ± 4.6	143.5 ± 3.1
Control at the end of 6-week experiment (n = 10)	431.2 ± 7.0 ***	375.2 ± 9.0 **	179.0 ± 5.1 *	168.0 ± 4.3 ***
4-week overtraining (n = 8)	345 ± 6.5 ###	322.1 ± 7.0 ###	163.2 ± 4.1 ##	151.3 ± 4.0 ##
6-week overtraining (n = 8)	308.7 ± 7.0 ### ** ¤¤	283.0 ± 6.8 ### ** ¤¤	152.2 ± 4.2 ##	150.1 ± 3.9 ##
6-week endurance training (n = 8)	412.3 ± 7.0 *** ¤¤¤ ∇∇∇	380.0 ± 9.5 *** ¤¤¤ ∇∇∇	184.0 ± 4.8 * ¤¤ ∇∇∇	170.5 ± 3.9 *** ¤¤ ∇∇

* — $p<0.05$; ** — $p<0.01$; *** — $p<0.001$ in comparison with control (10)
¤¤ — $p<0.01$; ¤¤¤ — $p<0.001$ in comparison with overtraining (28)
— $p<0.01$; ### — $p<0.001$ in comparison with control (42)
∇∇ — $p<0.01$; ∇∇∇ — $p<0.001$ in comparison with overtraining (42)

Table 2. Changes in the body and muscle growth rate during endurance and overtraining

Group	Body weight %/day	*Plantaris muscle* %/day	*Extensor digitarum longus muscle* %/day	*Soleus muscle* %/day
Control at the end of 6-week experiment (n = 10)	1.80 ± 0.08	1.05 ± 0.05	0.35 ± 0.04	0.58 ± 0.031
4-week overtraining (n = 8)	–0.37 ± 0.008 ***	–0.32.1 ± 0.006 ***	– 0.043 ± 0.0008 ***	0.28 ± 0.019 ***
6-week overtraining (n = 8)	– 1.11 ± 0.03 *** ¤¤¤	– 1.14 ± 0.02 *** ¤¤¤	– 0.29 ± 0.007 *** ¤¤¤	0.16 ± 0.05 *** ¤
6-week endurance training (n = 8)	1.35 ± 0.07 *** ¤¤¤ ###	1.17 ± 0.04 ¤¤¤ ###	0.47 ± 0.03 ¤¤¤ ###	0.64 ± 0.04 ¤¤¤ ###

*** — $p<0.001$ in comparison with control (42)
¤ — $p<0.05$; ¤¤¤ — $p<0.001$ in comparison with overtraining (28)
— $p<0.001$ in comparison with overtraining (42)

3. DEGRADATION OF CONTRACTILE PROTEINS DURING DIFFERENT VOLUMES OF ENDURANCE TRAINING

The increased degradation of muscle contractile proteins during both overtraining protocols is reflected by an increased daily proportion of 3MeHis excretion pool (Fig. 2). During 6 weeks of overtraining in fast-twitch muscles the content of DNA per muscle decreased: in Pla muscle to 133 ± 11.4 μg DNA/muscle (the control group 198.8 ± 18.1 μg DNA/muscle, $p<0.01$) and in EDL muscle 53.3 ± 0.6 (the control group 71.6 ± 0.8 μg DNA/muscle, $p<0.001$), which might indicate a decreased DNA synthesis rate in skeletal muscle but also loss of DNA because of loss of myonuclei during atrophy of muscle. At the same time DNA/protein ratio in Pla increased to 443 ± 14 in comparison with the control group (397 ± 12, $p<0.05$) and in EDL to 600 ± 16 (522 ± 15, $p<0.01$ in the control group), showing the amount of protein per nucleus. RNA/protein ratio had a tendency to decrease in all studied muscles.

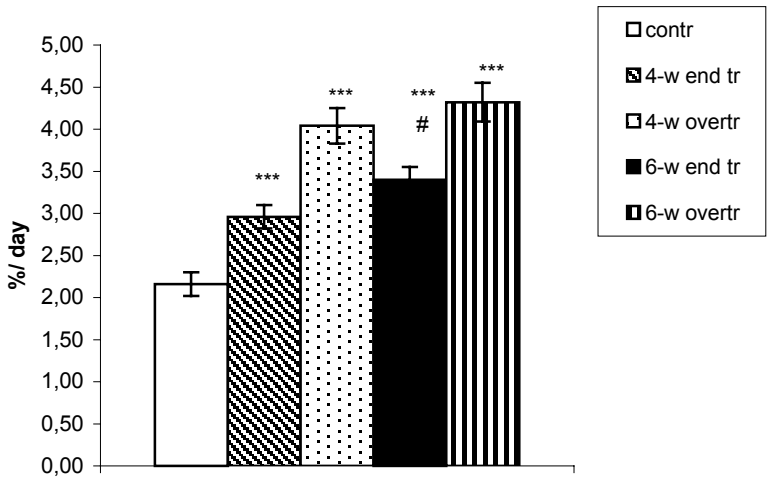

Figure 2. Changes of daily proportion of 3 MeHis pool excretion during endurance and overtraining contr — control group (n=10)
4-w end tr — rats were taken after 4-week endurance training and used as 4-week endurance training group for comparison with 4-week overtraining group (n=8)
4-w overtr — 4-week overtraining group. Rats of this group had 7 exercise-training days per week (n=8)
6-w end tr — 6-week endurance training group. Rats of this group had 5 exercise-training days per week (n=8)
6-w overtr — 6-week overtraining group. Rats of this group had 7 exercise-training days per week (n=8)

*** — $p<0.001$ in comparison with control group
\# — $p<0.05$ in comparison with 4-week endurance training group

4. THE EFFECT OF THE VOLUME OF ENDURANCE TRAINING ON THE SYNTHESIS RATE OF CONTRACTILE PROTEINS

The synthesis rate of MyHC during four and six weeks of overtraining decreased in all studied muscles (Fig. 3).

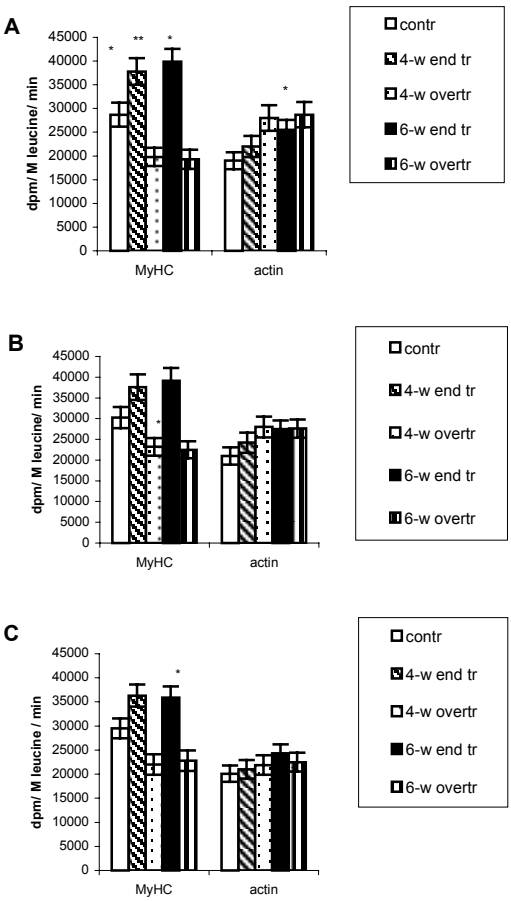

Figure 3. Changes in myosin heavy chain and actin synthesis rate 24 h after endurance and overtraining

A – *extensor digitorum longus* muscle
B – *plantaris* muscle
C – *soleus* muscle
contr — control group (n=10)
4-w end tr — rats were taken after 4-week endurance training and used as 4-week endurance training group for comparison with 4-week overtraining group (n=8)
4-w overtr — 4-week overtraining group. Rats of this group had 7 exercise-training days per week (n=8)
6-w end tr — 6-week endurance training group. Rats of this group had 5 exercise-training days per week (n=8)
6-w overtr — 6-week overtraining group. Rats of this group had 7 exercise-training days per week (n=8)

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

As shown in Fig. 4, MyHC isoforms' synthesis rate is different. MyHC I and IIa isoforms' synthesis rates are faster than others. The IIb isoform has the slowest synthesis rate both in Pla and EDL muscles (Fig. 4). Six weeks of overtraining did not change MyHC isoforms synthesis rate significantly in EDL muscle. In Pla muscle MyHC I isoform synthesis rate decreased during six weeks of overtraining from 33200 ± 2150 to 27100 ± 1800 dpm/ M leucine/min ($p<0.05$) and MyHC IIb from 20100 ± 1600 to 15500 ± 1400 dpm/ M leucine/min ($p<0.05$).

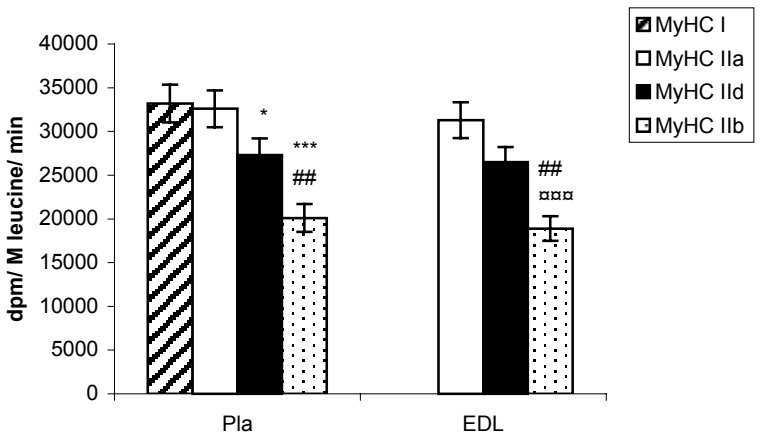

Figure 4. Myosin heavy chain isoforms' synthesis rate
Pla — *plantaris* muscle of control group
EDL — *extensor digitorum longus* muscle of control group
* — $p<0.05$
*** — $p<0.001$
} in comparison with MyHC I isoform
— $p<0.01$ in comparison with MyHC IId isoform
¤¤¤ — $p<0.001$ in comparison with MyHC IIa isoform

MyHC synthesis rate 24 h after the last endurance training increased in the endurance trained group and decreased in the overtraining group. MyLC isoforms synthesis rate did not change significantly (Fig. 5A) during exercise training.

Actin synthesis rate increased in all groups. Tropomyosin synthesis had the tendency to increase in all groups and Tn-T synthesis decreased in the overtrained group. Tn-I synthesis increased in the overtrained group (Fig. 5B) as well as α-actinin (Fig. 5C). C-protein synthesis rate decreased in the overtrained group (Fig. 5C).

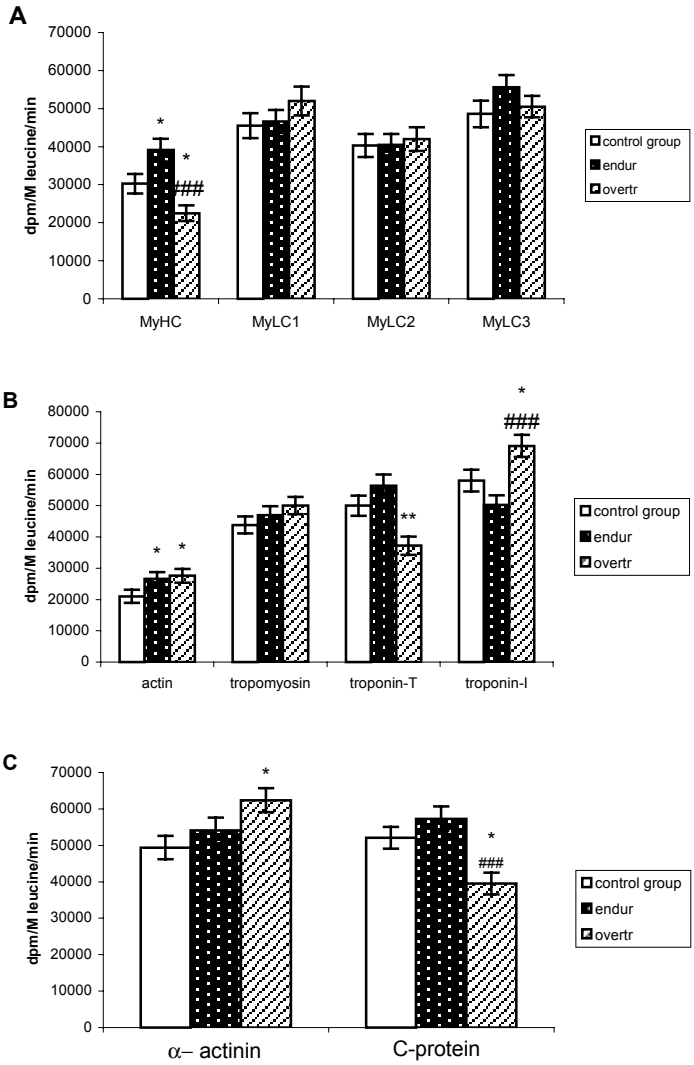

Figure 5. The effect of training volume on the synthesis rate of myofibrillar proteins in *plantaris* muscle

A — myosin
B — actin and regulatory proteins
C — minor proteins
contr — control group
endur — endurance trained group
overt — overtrained group
* — $p<0.05$ in comparison with control group
— $p<0.001$ in comparison with endurance trained group

5. THE EFFECT OF THE VOLUME OF ENDURANCE TRAINING ON THE RELATIVE CONTENT OF MyHC ISOFORMS

It is surprising that during overtraining, MyHC I isoform relative content decreased (Fig. 6). The relative content of MyHC IIa isoform increased in Pla muscle during 6 weeks of overtraining (Fig. 6).

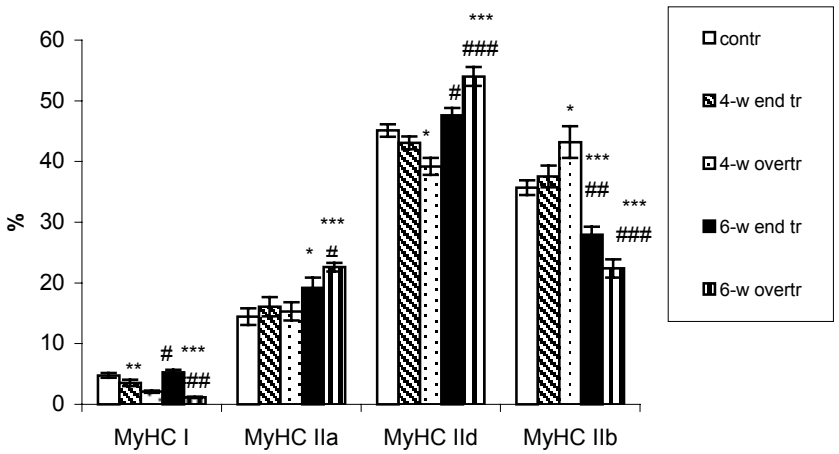

Figure 6. **The effect of endurance and overtraining on myosin heavy chain isoforms' composition in *plantaris* muscle**

contr — control group (n=10)

4-w end tr — rats were taken after 4-week endurance training and used as 4-week endurance training group for comparison with 4-week overtraining group (n=8)

4-w overtr — 4-week overtraining group. Rats of this group had 7 exercise-training days per week (n=8)

6-w end tr — 6-week endurance training group. Rats of this group had 5 exercise-training days per week (n=8)

6-w overtr — 6-week overtraining group. Rats of this group had 7 exercise-training days per week (n=8)

* — $p<0.05$
** — $p<0.01$
*** — $p<0.001$
} in comparison with control group

\# — $p<0.05$
— $p<0.01$
— $p<0.001$
} in comparison with subsequent 4-week training group

In Pla muscle relative content of MyHC IId isoform relative content decreased during 4 weeks of overtraining and increased during 6 weeks of overtraining (Fig. 6). The relative content of MyHC IIb isoform increased in Pla muscle during 4 weeks of overtraining and decreased during 6 weeks of overtraining (Fig. 6).

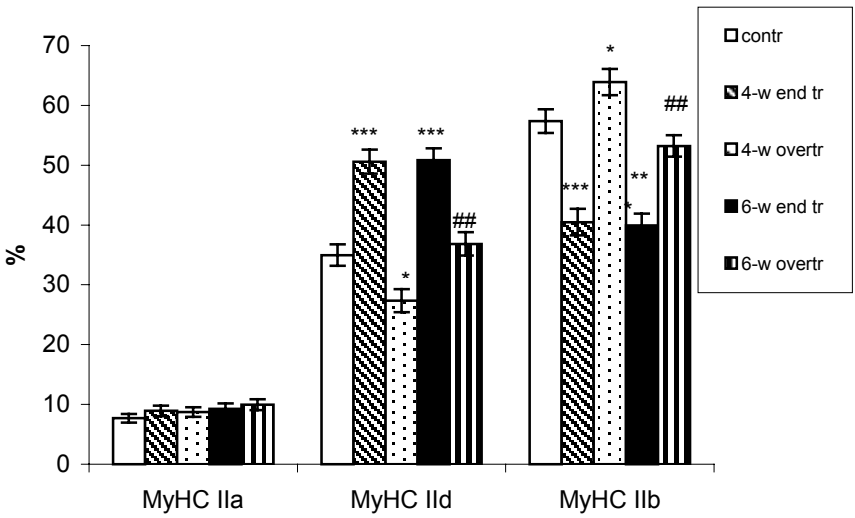

Figure 7. The effect of endurance- and overtraining on myosin heavy chain isoforms' composition in *extensor digitorum longus* muscle

contr — control group (n=10)

4-w end tr — rats were taken after 4-week endurance training and used as 4-week endurance training group for comparison with 4-week over-training group (n=8)

4-w overtr — 4-week overtraining group. Rats of this group had 7 exercise-training days per week (n=8)

6-w end tr — 6-week endurance training group. Rats of this group had 5 exercise-training days per week (n=8)

6-w overtr — 6-week overtraining group. Rats of this group had 7 exercise-training days per week (n=8)

* — $p<0.05$
*** — $p<0.001$
} in comparison with control group

\# — $p<0.05$
— $p<0.01$
} in comparison with subsequent 4-week training group

In EDL muscle the relative content of MHC IIb isoform increased during 4 weeks of overtraining (Fig. 7). In Sol muscle the relative content of MyHC I isoform did not change significantly during overtraining.

6. RELATIONS BETWEEN MYOFIBRILLAR PROTEINS, ENDURANCE TRAINING VOLUME AND PWC

Among myofibrillar proteins the decrease of the synthesis rate of protein C, troponin-T and MyHC had the most significant negative correlation with increasing training volume (subsequently $r= -0.727$, $p<0.001$; $r= -0.713$, $p<0.001$ and $r= -0.537$, $p<0.05$).

MyHC I and IIb isoforms in FT muscle are most sensitive to the increase of training volume. There is also negative correlation between the increase of training volume and the above mentioned MyHC isoforms relative content. MyHC I ($r= -0.817$, $p<0.001$) and MyHC IIB ($r= -0.802$, $p<0.001$).

There are also significant correlations between the PWC and the synthesis rate of MyHC ($r=0.687$, $p<0.001$), protein C ($r=0.664$, $p<0.001$) and MyHC I isoform relative content ($r =0.612$, $p<0.001$).

7. REFLECTION OF EXERCISE MYOPATHY ON THE ULTRASTRUCTURE OF OXIDATIVE-GLYCOLYTIC MUSCLE FIBRES

In the myopathic FT muscle fibres the most typical ultrastructural changes are the destruction of peripheral myofilaments, attenuation of myofibrils and complete destruction of some sarcomeres (Fig. 8).

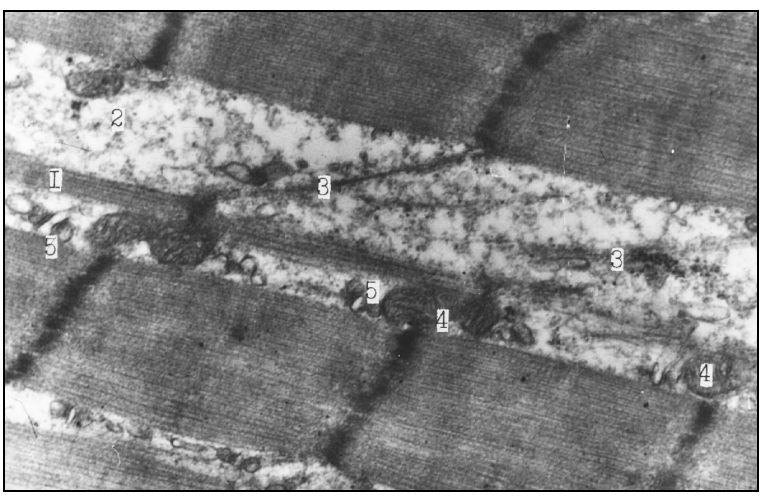

Figure 8. Electron micrograph of a fragment of fast-oxidative-glycolytic muscle fibre in the state of contraction. Almost all of the myofibril is destructed (1) and in the result wide intermyofibrillar space has formed (2). Irregularly located thick myofilaments in the sarcoplasma (3), mitochondria (4), trads (5). Magnification 32 000x.

Due to the destruction of myofibrils the sarcoplasm-filled spaces between myofibrils increase. These spaces contain mitochondria in which can be seen a quantity of cristae and a compact matrix, fragments of T-tubules, fragments of the sarcoplasmic reticulum and a quantity of glycogen granules. In some myofibrils warped Z-lines can be observed (Fig. 9).

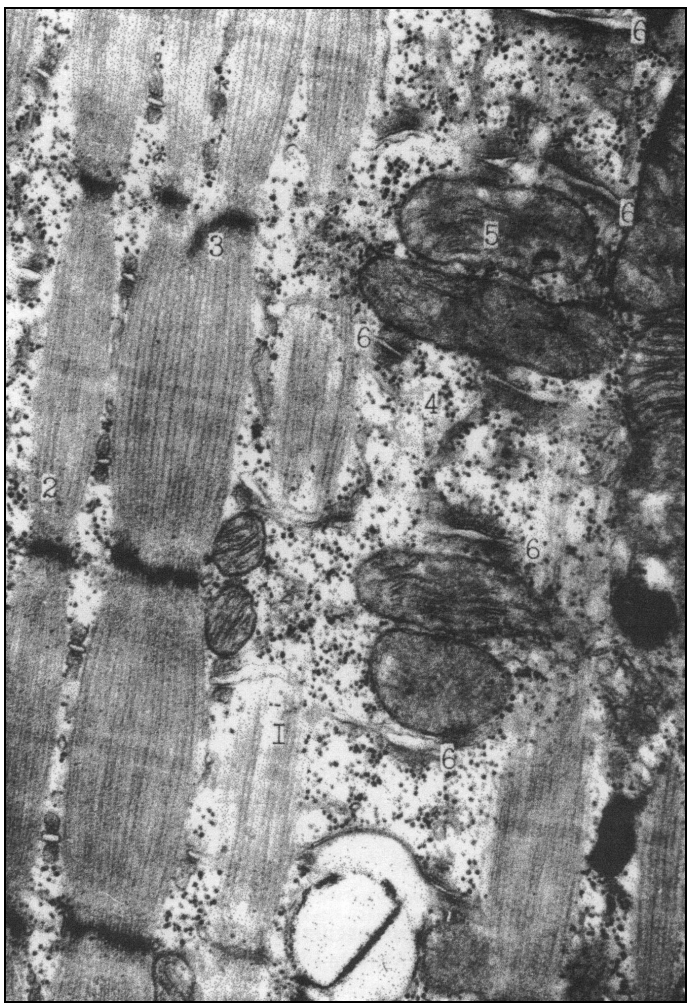

Figure 9. Electron micrograph of fragment a of fast-oxidative-glycolytic muscle fibre. Destructions of myofibrils (1), thin myofibrils (2), damage of the Z-line (3), a quantity of glycogen granules in the wide intermyofibrillar space (4), mitochondria on both sides of Z-line (5), T-tubules on the level of the border of A and I discs (6).
Magnification 27 000x.

The destruction of muscle fibre organelles is accompanied by the activation of lysosomal structures. Long-term muscle exertion causes a decrease in the osmosis of lipid inclusions which is most probably connected with the more intensive use of fatty acids as substrate of energy (Fig. 10).

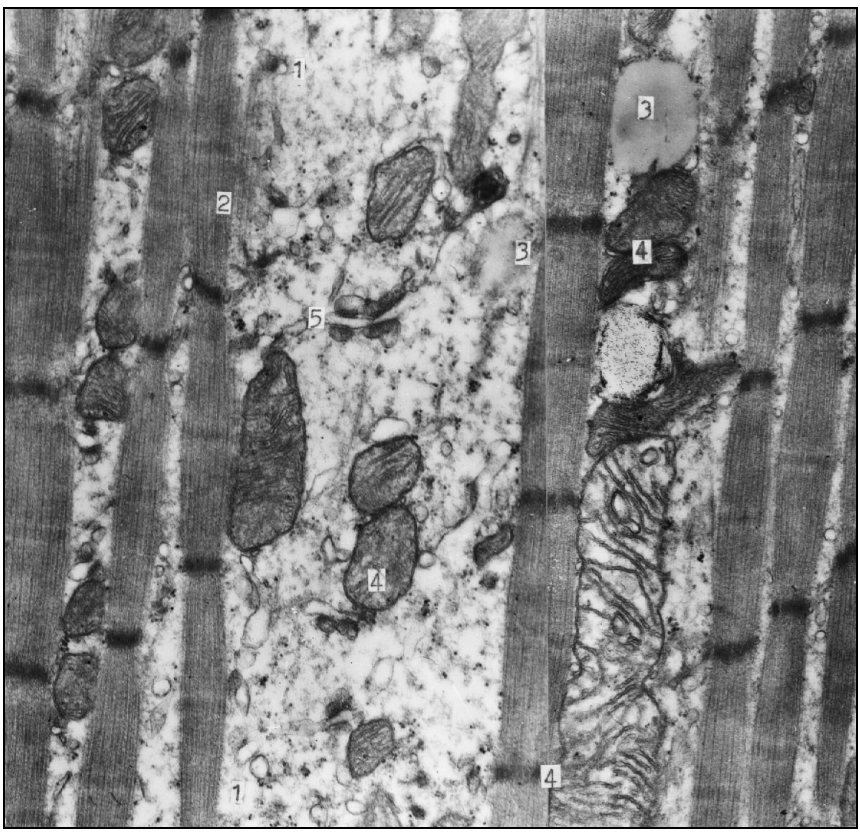

Figure 10. **Electron micrograph of a fragment of fast-oxidative-glycolytic muscle fibre.** Wide intermyofibrillar space due to destruction of myofibrils (1), thin myofibrils (2), lipid droplets (3), located among mitochondria (4), and T-tubules (5).
Magnification 26 000x.

In the fast-oxidative-glycolytic (F-OG) muscle fibres there also occur some satellite cells. Under the light microscope there emerges a clear tendency towards increasing the number of nuclei in the muscle fibres that contain 2–3 nucleoli. The electron microscopic studies confirm this.

More extensive and more clearly expressed destructive changes occur in the exercise caused myopathic F-OG muscle fibres bringing about atrophy of those

muscle fibres. At the same time those muscle fibres reveal morphological symptoms of the continuation of restoration processes: increase in the number of nucleoli, satellite cells under the basal membrane, activation of the mitochondrial system, the occurrence of numerous lipid drops and glycogen granules.

8. SIMILARITIES BETWEEN EXERCISE AND GLUCOCORTICOID MYOPATHIES

The ultrastructural study in corticosteroid myopathic muscle showed the disarray of thick myofilaments in FT G fibres in dexamethasone-treated rats. Obviously the destructive process of myofilaments begins from the periphery of myofibrils, spreads to the central part of sarcomere near the H-zone and is distributed over all the A-band (Fig. 11).

Figure 11. **Disarray of thick myofilaments in fast glycolytic type of muscle fibres** Magnification 52 333x.

We have got the impression that the thin filament and Z-line are much more resistant to any catabolic action of dexamethasone than thick filaments in G muscle fibres (Fig. 11). The myofibrils of G muscle fibres are thinner in dexamethasone-treated rats. This is caused by the splitting of myofibrils (Fig. 12). In OG fibres the destruction of thick myofilaments was remarkably less pronounced. Myofibrils were structurally normal in the dexamethasone-treated Sol muscle. There was evidence of increased lysosomal activity in the G muscle fibres and in the satellite

cells of dexamethasone-treated rats. This manifested itself in increased numbers of lysosomes, autophagic vacuoles, which contain mitochondria and other membranous structures. In G fibres from the corticosteroid-treated rats the sarcoplasmic reticulum was frequently more prominent than in the controls. There were only slight morphological changes in OG fibres and there no structural changes in ST muscle fibres during hormone treatment.

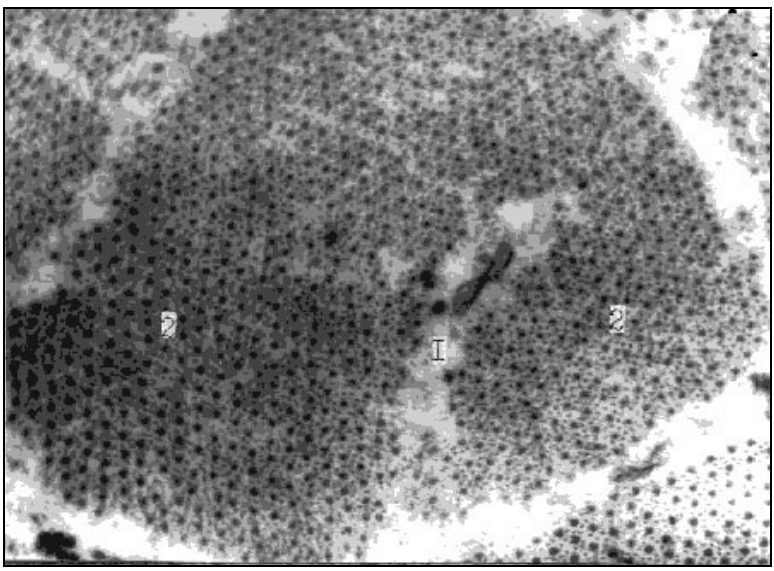

Figure 12. Splitting of myofibrils
Magnification 40 333x.

9. CATABOLIC ACTION OF GLUCOCORTICOIDS

The main reason for the development of muscle atrophy in corticosteroid myopathy is the accelerated catabolism of muscle proteins. It is well established that lysosomal and non-lysosomal pathways also exist in skeletal muscle to account for the degradation of their intracellular proteins. As the content of lysosomes in skeletal muscle is relatively low, the non-lysosomal pathway makes a particularly significant contribution and may be of special importance in the initial rate-limiting steps in the catabolism of myofibrillar proteins and, consequently, in the regulation of their turnover rate.

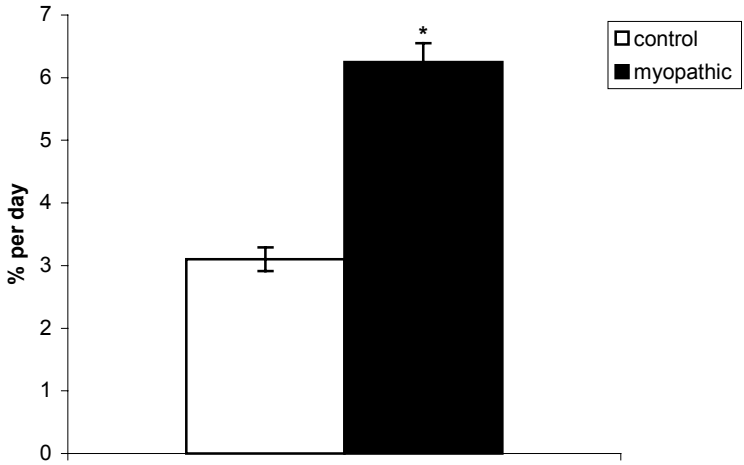

Figure 13. Contractile proteins degradation
* — p<0.01 in comparison with control group

A promoted breakdown of contractile proteins myosin and actin (Fig. 13) in the muscle during glucocorticoid administration was also shown by the enhanced excretion of 3-methylhistidine (Table 3.). The ST skeletal muscles are much more resistant than the FT muscles to any catabolic action of the corticosteroids.

Table 3. Characteristics of degradation in skeletal muscle

Measures	Control	Myopathic
Body weight, g	264 ± 6	226 ± 7
Muscle mass, g/rat	118.8 ± 3.9	101.0
Myofibrillar protein, g/rat	15.8 ± 0.8	12.0 ± 0.5
Total 3-MeHis in skeletal muscle, μmol/rat	95.18 ± 2.31	72.9 ± 1.96
Daily excretion of 3-MeHis, %	2.0 ± 0.12	5.9 ± 0.3

10. THE ROLE OF ALKALINE PROTEINASE IN INTRACELLULAR CATABOLISM

The catabolic effect of glucocorticoids on myofibrils seems to be realised through the augmented alkaline proteinase activity (Fig. 14). The weight reduction of skeletal muscle mass in dexamethasone-treated animals is in full accordance with the augmentation of their alkaline protease activity (Fig. 14, Table 3). Although a significant weight reduction occurred only in the fast-glycolytic (F-G) fibres, the intracellular catabolic effect of glucocorticoids was noted in the fast OG and slow-oxidative (S-O) muscle fibres as well. Alkaline proteases are synthesized in mast cells. After degranulation the enzyme enters the muscle cell (Fig. 15), but the mechanism is unknown. Upon administration of large doses of glycocorticoids there is an increase in the number of mast cells in the perivascular porous connective tissue of the muscle fibres. Around the fast glycolytic muscle fibres, degranulation of mast cells is very clearly expressed. Simultaneously the number of mast cells in the lymph node medulla is considerably decreased. The lymph nodes are probably the sources of the muscle mast cells. This may imply that the increased number of mast cells may be the result of their migration from the lymph nodes.

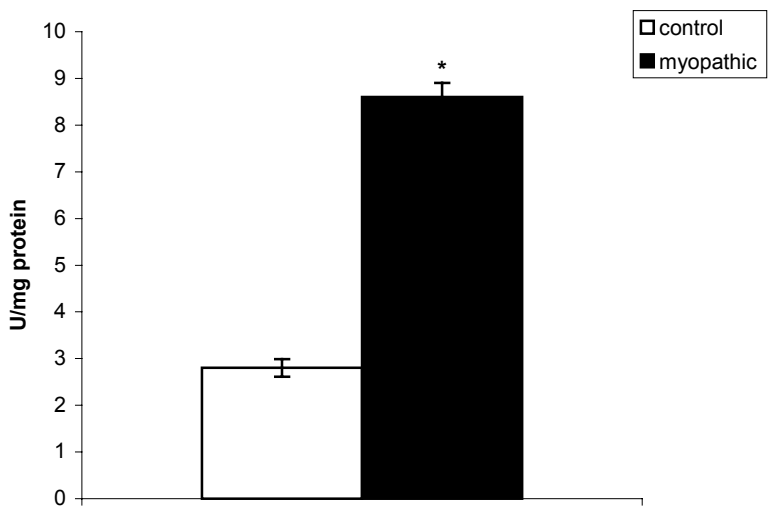

Figure 14. Alkaline proteinase activity
* — $p<0.01$ in comparison with control group

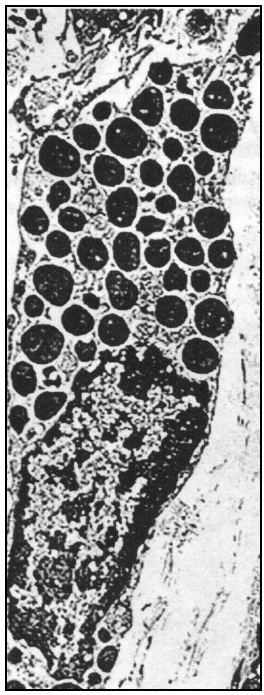

Figure 15. Degranulation of mast cell

11. CHANGES IN DNA IN SKELETAL MUSCLE

Basic characteristics of glucocorticoid caused myopathy like the decrease in body weight, muscle strength and motor activity are presented in Table 4. In Table 5 are characterized changes in different muscles during the glucocorticoid treatment. The decrease in the fast-twitch muscle weight, DNA synthesis and muscle size were determined by the number of DNA-units. The increase in DNA-unit size shows the decrease in the total muscle protein synthesis, and the decrease in RNA-unit size shows the decrease in RNA concentration in fast-twitch muscles in glucocorticoid treated rats.

Grip strength of forelimb and hindlimb and daily motor activity of rats were used for characterization of development of glucocorticoid caused myopathy. Ambulatory activity characterizes movemental activity of animals, total activity includes stereotypic (scratching, grooming, digging...) non-ambulatory movements.

Table 4. The effect of dexamethasone on the body weight, muscle strength, motor activity

	Body weight (g)	Grip strength (N)		Daily motor activity (movements/h)	
		Forelimb	Hindlimb	Total	Ambulatory
Before (n=10)	253.0 ± 5.6	9.52 ± 0.40	15.54 ± 0.86	933 ± 42	482 ± 23
After (n=10)	200.0 ± 5.1 ***	6.19 ± 0.35 ***	8.55 ± 0.70 ***	559 ± 31 ***	266 ± 18 ***

Before — before dexamethasone treatment
After — after 10 days dexamethasone treatment

*** — $p<0.001$ in comparison with before dexamethasone treatment

3MeHis – daily 3-methylhistidine pool excreted was used for characterization of contractile proteins degradation.

Table 5. The effect of dexamethasone on the muscle weight and content of DNA and RNA in different muscles

Group		*Extensor digitorum longus* muscle	*Plantaris* muscle	*Soleus* muscle
Control (n=10)	weight (mg)	98.0 ± 3.2	192.0 ± 5.6	86.0 ± 2.9
	DNA unit number	39.2 ± 1.9	101.8 ± 3.1	67.9 ± 2.8
	DNA unit size	523 ± 32	396.0 ± 25	268.0 ± 22
	RNA unit size	4.31 ± 0.28	4.29 ± 0.19	6.38 ± 0.33
	RNA/DNA	2.30 ± 0.2	1.70 ± 0.1	1.71 ± 0.1
Myopathic (n=10)	weight (mg)	57.0 ± 2.9 ***	126.0 ± 3.8 ***	81.0 ± 3.4
	DNA unit number	17.1 ± 0.8 ***	50.4 ± 2.4 ***	61.6 ± 3.7
	DNA unit size	702.0 ± 53 **	526.0 ± 26 **	291.6 ± 21
	RNA unit size	2.85 ± 0.2 ***	3.09 ± 0.2 ***	6.05 ± 0.3
	RNA/DNA	2.60 ± 0.2	1.62 ± 0.1	1.76 ± 0.2

DNA unit number — DNA content μg/muscle
DNA unit size — protein/ DNA (mg/mg)
RNA unit size — RNA/ protein (μg/mg)
RNA/DNA — RNA/DNA (μg/μg)
DNA — unit is imaginary volume of cytoplasm managed by a single nucleus. DNA — unit is therfore only an operational term.
RNA unit size expresses muscle RNA concentration.

12. CHANGES IN MyHC ISOFORMS SYNTHESIS RATE

Changes in MyHC isoforms' synthesis rate in glucocorticoid myopathic skeletal muscles are shown in this study for the first time. The synthesis rate of MyHC I isoforms does not depend on the twitch characteristics of the skeletal muscle (Fig. 16A).

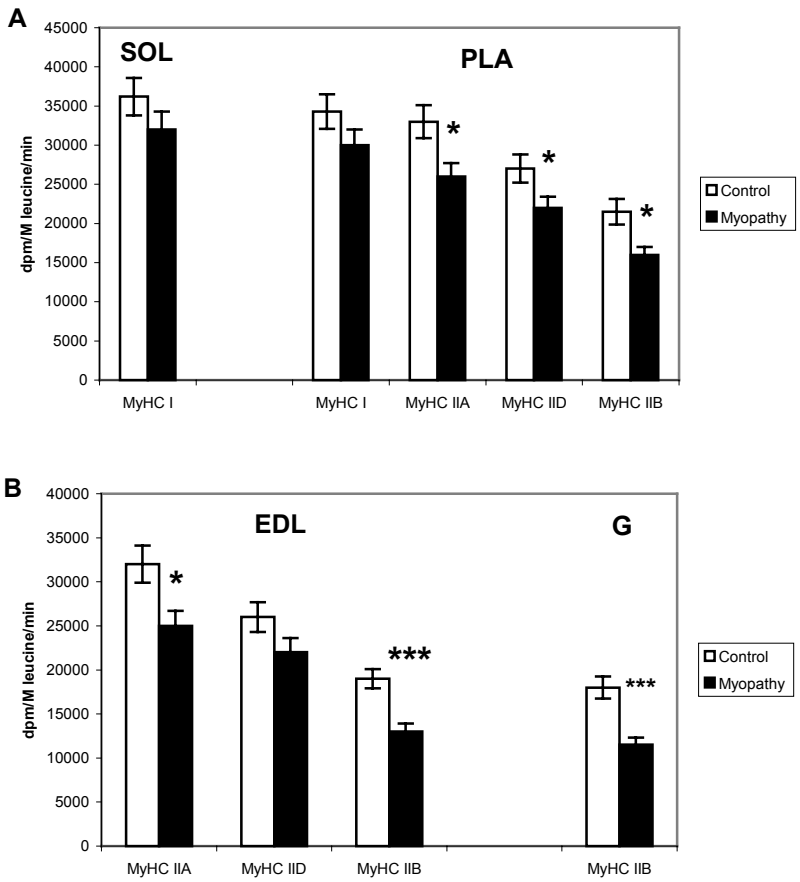

Figure 16. Changes in MyHC isoforms synthesis rate in different muscles in glucocorticoid myopathy

SOL— *soleus* muscle, PLA — *plantaris* muscle (A), EDL — *extensor digitorum longus* muscle, G — fast-twitch glucolytic muscle fibres from *quadriceps femoris* muscle (B)

* — $p<0.05$

*** — $p< 0.001$

} in comparison with subsequent control group

In both studied fast-twitch muscles the synthesis rate of MyHC type II isoforms decreased, as well as in fast-twitch glycolytic muscle fibres (Fig.16A, B). Therefore, the synthesis rate of MyHC IIb isoforms decreased more significantly than the rate of IIa and IId (Fig. 16A, B).

13. CHANGES IN MyHC RELATIVE CONTENT

In Sol muscle MyHC isoform composition did not change siginificantly as well as in PLA muscle (Fig. 17). As seen in Fig. 17 and 18, the relative content of MyHC IIb isoform decreased in both studied muscles and in glycolytic muscle fibres, and the relative content of IIa and IId isoforms increased at the same time.

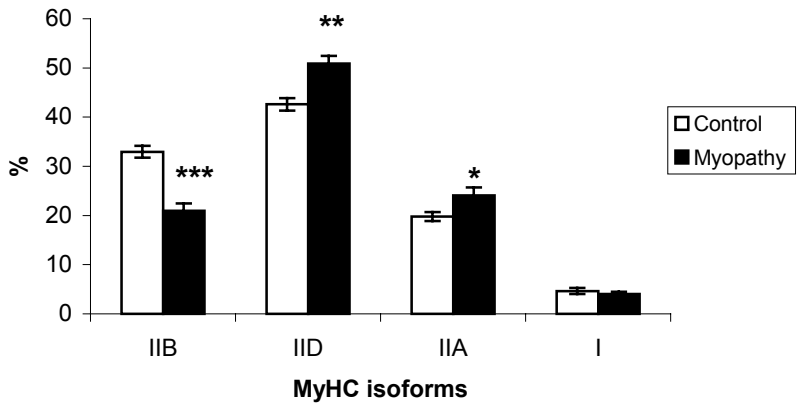

Figure 17. Changes in MyHC isoforms relative content in *plantaris* muscle in glucocorticoid myopathy

* — p<0.05
*** — p< 0.001
} in comparison with subsequent control group

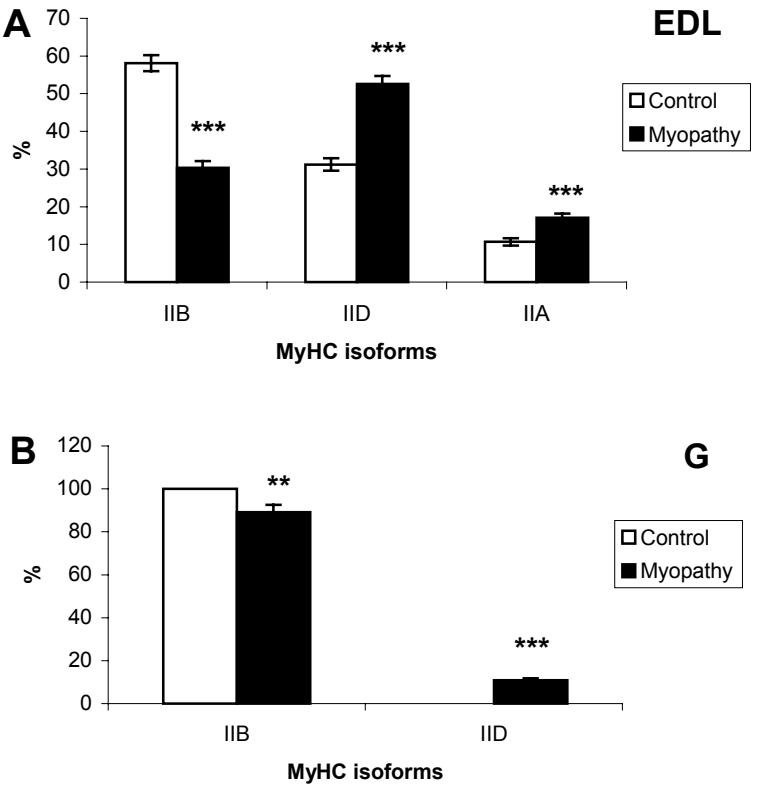

Figure 18. **Changes in MyHC isoforms relative content in *extensor digitorum longus* muscle (A) and glycolytic muscle fibres (B) in glucocorticoid myopathy**

** — $p<0.05$
*** — $p< 0.001$
} in comparison with subsequent control group

14. SENSITIVITY OF MyHC ISOFORMS TO THE ALKALINE PROTEINASE

Incubation of myofibrils from all the studied muscles with alkaline proteinase shows that the sensibility of MyHC isoforms under *in vitro* conditions depends on muscle twitch and metabolic characteristics as well as on the type of isoform itself. In Pla muscle MyHC IIB isoform decreased during incubation with alkaline proteinase 9 ± 0.9%, changes in MyHC IId, IIa and I isoform were not significant. In EDL muscle MyHC IIb isoform content decreased 12 ± 0.9%,

changes in other MyHC isoforms in this muscle were statistically insignificant. In glycolytic muscle fibres the content of MyHC IIb isoform decreased during incubation with alkaline proteinase 15 ± 1.1%. The comparison of MyHC IIB isoform decrease in Pla and EDL muscle ($p<0.05$) and in glycolytic muscle fibres ($p<0.001$) shows that sensibility of MyHC IIB isoforms to alkaline proteinase depends on the muscle oxidative potential.

Changes in MyHC I isoforms in Pla and Sol muscles during incubation were about 2% and statistically insignificant. Comparison of changes in myosin turnover rate in myopathic muscle show that only the turnover rate of MyHC is decreasing (Fig. 19).

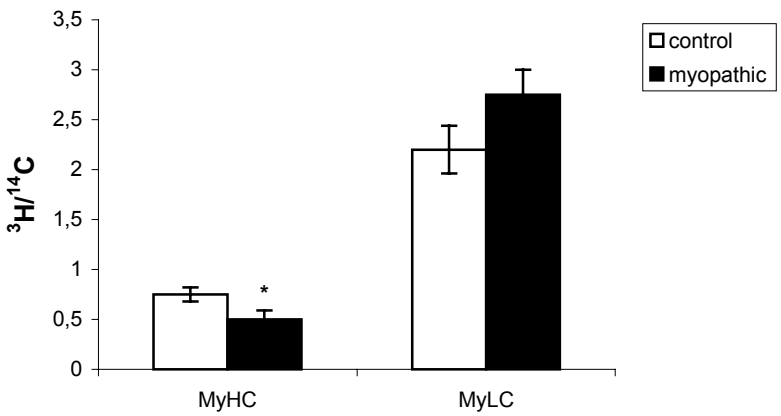

Figure 19. Myosin turnover rate in fast glycolytic type of muscle fibres
* — $p<0.05$ in comparison with control group

DISCUSSION

THE EFFECT OF TRAINING VOLUME ON THE PHYSICAL WORKING CAPACITY

Skeletal muscle fibres exist as dynamic structures and are capable of adapting to altered functional demands. The structural heterogeneity of muscle tisue reflects its high degree of functional specialization and is the basis of its plasticity (Pette & Staron, 1990). Different muscle fibres are recruited during exercise in dependance of intensity and duration of exercise. Among FT fibres OG muscle fibres are unique in this respect and, at least partially, this is caused by the wide range of recruitment of these fibres during physical activity, including endurance training (Seene & Umnova, 1992). Endurance training is characterized by its high volume. A particularly important role in endurance training is played by the increase of training volume (Viru & Viru, 1999)

As our experiment showed, fast increase of training volume exceeding by 3–4 times that of the training protocol, was leading to overtraining already during four weeks of exercise training. During the fifth and sixth weeks daily training volume decreased as rats were not able to keep higher training volume, but the overtraining syndrome deepened. After 4 weeks of overtraining PWC decreased by 38% and after 6 weeks of overtraining by 54% in comparison with the endurance training protocol. It is widely accepted that the universal finding during overtraining is a decrease in performance ability. It is obvious from this work that fast increase of the training volume and lack of recovery in the overtraining groups exercise protocols was leading to overtraining. Problems of recovery seem to appear when the exercise training time is reaching 10% of the twenty-four hours. Significant decrease of PWC in the overtraining groups, in comparison with endurance training group, shows that these training protocols were leading to overtraining. Training stimulus in the overtraining groups lasted for too long in each exercise session and was too frequent, which interrupted the recovery phase and the necessary adaptation did not occur.

One reason why top athletes make mistakes with using high exercise volume is that progressive overload is the foundation of successful endurance training (Jensen et al., 1997). Probably exercise training volume and its fast increase are only a part of the reasons leading to the overtraining syndrome. For example, the importance of recovery is shown by the fact that when during the third week of the overtraining program we decreased training volume one day by 60%, the next day the rats were able to tolerate 150% of the prior exercise volume. During the fourth week of overtraining when we decreased the training volume again by 60%, the next day the rats tolerated 110% of the prior exercise volume. Further decrease of the training volume did not give any effect.

CHANGES IN SKELETAL MUSCLE CONTRACTILE PROTEINS IN OVERTRAINING

The DNA content in muscle, protein and DNA ratio in FT muscles are decreasing during overtraining, showing signs of overtraining caused myopathy as a result of muscular overload. As far as is known at present, overtraining myopathy is characterized by slow turnover of MyHC in FT muscle fibres, depressed neuromuscular and depressed alpha-motoneuron excitability.

The decreased synthesis and increased degradation rate of contractile proteins that we observed in overtrained muscles are in good agreement with the increased occurrence of destructive processes in FT fibres.

Contrary to decreased turnover rate of contractile proteins, overtrained athletes show a persistent high synthesis rate or concentration of heat shock proteins during exercise training, which might show the increased stress tolerance of affected cells and conduct cellular repair process (Salo *et al*, 1991).

Muscle fibre phenotype maintenance and transition depends on motoneuron-specific impulse patterns, neuromuscular activity and mechanical loading. Depending on the type, intensity, and duration of changes in any of these factors, muscle fibres adjust their phenotype to meet the altered functional demands (Pette, 2001).

It is known that muscle protein content and quality depends on the integrity of the remodeling process of muscle. The remodeling involves removal of an old protein and replacement with new. Synthesis of mixed muscle protein may not reflect changes occurring in individual proteins (Balagopal *et al*, 1997), as well as MyHC synthesis rate may not reflect changes in different MyHC isoforms synthesis rate.

It is shown in this study that MyHC I and IIa isoforms' synthesis rate is faster than synthesis rate of other MyHC isoforms and synthesis rate MyHC IIb isoform is the lowest. The *soleus* muscle resistance to overtraining may be related with its MyHC composition. It contains about 1% MyHC IIa isoforms, the MyHC I isoform is makes up about 99% of the MyHC. At the same time MHC I isoform synthesis rate and relative content are decreasing in FT Pla muscle and MyHC IIa and IId isoforms' percentage is increasing during 6-week overtraining in this muscle.

It seems that MyHC isoforms' relative content during the overtraining syndrome depends on the duration, volume of exercise and type of muscle. Training volume increase by 185% in comparison with control group during 4-week overtraining and by 285% during 6-week overtraining, decreased PWC subsequently by 38% and 55% and caused different changes in the relative content of MyHC isoform in FT muscles. Decrease of aerobic working capacity during increase of excessive training volume was accompanied by decrease of MyHC I isoform relative content in FT muscle. This might indicate that the type I and IIa MyHC isoforms are less stable than the type IId and IIb MyHC isoforms in FT muscles during high volume exercise. As was mentioned above, activity

pattern of the fibres, where MyHC I and IIa isoforms are dominant, have relatively high oxidative potential and are more recruited during endurance type of exercise. These fibres are also more susceptible to oxidative damage by reactive oxygen species than fibres where MyHC IIb and IId isoforms are prevailing.

This higher stability of the MyHC IIb isoform in FT muscles may also explain why the MyHC IIb isoform content initially increases during overtraining despite the reduced synthesis rate. The logic of changes in MyHC pattern during overtraining does not act similarly during endurance training. There may be several reasons for changes in MyHC composition in FT skeletal muscles in overtraining syndrome, but hypoxia and ischemia (Vescovo *et al,* 1998; Bigard *et al*, 2000) have been also shown to cause increase of fast MyHC isoforms in skeletal muscle and during chronic heart failure ST fibres tend to be replaced by FT fibres (Coskar *et al*, 2000). These reasons may play a certain role in case of 4-week overtraining as well.

Exercise intolerance in overtraining syndrome is localized in skeletal muscle as decreased capillarization may impair the exchange of oxygen between capillaries and muscle tissue and this may contribute to exercise intolerance (Degens *et al*, 2002). This might explain changes in slow- to fast MyHC isoform pattern during overtraining in FT skeletal muscle.

THE EFFECT OF TRAINING VOLUME ON THE REGULATORY AND MINOR PROTEINS

Functional changes in FT muscles may be related with differences in oxidative potential between muscles (Seene & Umnova 1992). Decrease of the synthesis rate of MyHC, Tn-T and C-protein during excessive increase of training volume, and significant correlation between changes of the above mentioned myofibrillar proteins and training volume, show that changes in MyHC isoforms during increased training volume may be only a part of the complex of changes in different individual myofibrillar proteins and their isoforms. There may be several reasons for changes in MyHC composition in skeletal muscles during excessive training volume, but hypoxia (Bigard *et al*, 2000) and ischemia (Vescovo *et al,* 1998) have been shown to cause increase of fast MyHC isoforms in skeletal muscle, and during chronic heart failure ST fibres tend to be replaced by FT fibres (Coskar *et al*. 2000). In the present work it was also shown that the decrease of aerobic working capacity during excessive training volume was accompanied with the decrease of MyHC I isoforms' relative content in FT skeletal muscle. The significant functional role of C-protein during the increase of training volume also shows the mathematical approach in the present study. The partial correlations after elimination of the effect of C-protein were calculated. It appears that the synthesis rate of MyHC which had the negative correlation with increasing training volume ($r=-0.537$, $p<0.05$), lost the significance in conditions where the role C-protein was eliminated. This

proves once again the functional significance of C-protein in muscle contraction in the conditions of excessive increase of training volume. C-protein seems to play an essential role in correct thick filament formation during myofibrillogenesis, modulating muscle contraction, and increasing the maximum shortening velocity (Hofmann *et al,* 1991). C-protein can either bind actin and myosin or affect the mechanical properties of myosin cross-bridges by linking the S2 segment of myosin to the backbone of the thick filament (Hofmann *et al,* 1991). The Z-disc protein α-actinin and regulatory proteins Tn-I and TM seem to be more resistant to excessive training volume. Regulatory protein Tn-T, like minor C-protein is very sensible to high training volume and together with MyHC isoforms may play the key role in changes of function properties of contractile machinery during the period of excessive training volume.

THE CORTICOSTEROID MYOPATHY

Contractile properties of skeletal muscle depend on the composition of MyHC isoform in the muscle. Unfortunately, changes in MyHC isoforms' composition and turnover rate during glucocorticoid myopathy have been poorly studied.

Skeletal muscle weakness in case of glucocorticoid myopathy has been caused by lesions of the myofibrillar apparatus and by changes in the neuromuscular synapses, particularly in FT G muscle fibres (Seene *et al*, 1988). However, it is unclear how the catabolic effect of glucocorticoids, particularly selective degradation of contractile proteins, including MyHC isoforms, is realised in the skeletal muscle. It is well established that lysosomal and non-lysosomal pathways exist in the skeletal muscle to account for the degradation of their intracellular proteins. As the content of the lysosomes in the skeletal muscle, particularly in FT muscles, is relatively low, the non-lysosomal pathway makes a particularly significant contribution and may be of special importance in the initial rate-limiting steps in the catabolism of myofibrillar proteins.

At the same time the synthesis rate of contractile proteins, particularly in FT muscles and G fibres, decreases. It has been shown that glucocorticoid treatment decreases the synthesis rate of MyHC (Seene, 1994; Hickson *et al*, 1995). The results of this study support the above mentioned standpoint. The changes in the synthesis rate of different MyHC isoforms in glucocorticoid caused myopathic skeletal muscle have been shown in this study for the first time. The synthesis rate of MyHC I isoform does not change in myopathic soleus muscle where this isoform makes up about 99% of MyHC, as well as in plantaris muscle where it forms about 5% of MyHC. In myopathic muscles the synthesis rate of type II MyHC isoforms decreases. In glycolytic fibres the expression of MyHC IId isoform synthesis was initiated in myopathic muscles. It has been also shown earlier in the diaphragm that dexamethasone treatment decreased MyHC IIb isoform expression (Prezant *et al*, 1998). It appears from our experiment that the synthesis rate of MyHC isoforms in myopathic muscles

does not depend so much on metabolic and twitch characteristics of the muscle, but mainly on the type of MyHC.

It was shown about two decades ago that MyHC are more sensitive to the action of alkaline proteinases than MyLC, at least under *in vitro* conditions (Seene & Alev, 1985). The glucocorticoid treatment increased the activity of alkaline proteinase in the skeletal muscle (Mayer *et al*, 1980; Seene & Viru, 1982). Alkaline proteinases are synthesized in mast cells (Seene, 1994). After degranulation the enzyme enters the muscle fibre. Upon administration of large doses of glucocorticoids there is an increase in the number of mast cells in the perivascular porous connective tissue of the muscle fibres (Seene, 1994). The degranulation of mast cells is clearly expressed around the F-G muscle fibres (Hussar *et al*, 1992). The lymph nodes are the sources of the muscle mast cells, which may be migrated from there. In myopathic muscles where the alkaline proteinase activity is high, the G fibres separate from the adjacent ones, bend and lyse. As the results of the present study show, the incubation of myofibrils from the muscles of different metabolic and twitch characteristics with alkaline proteinases, the sensibility of MyHC IIb isoforms depend to some extent on the above mentioned characteristics of the muscle. In FT muscles, where the oxidative potential is higher, the sensibility of MyHC IIb isoform to the alkaline proteinase activity is diminished.

Taking into account the synthesis rate of MyHC isoforms and their sensibility to alkaline proteinases, changes in the relative content of MyHC isoforms in our study in different muscles seem logical enough. As a result of the decrease in the synthesis rate of MyHC IIb isoform and its increased degradation, the relative content of this MyHC IIb isoform decreases in myopathic skeletal muscle.

The synthesis rate of MyHC IIb isoform in case of glucocorticoid — caused myopathy decreases in skeletal muscles in which the oxidative potential is low. At the same time the sensibility of MyHC IIb isoform to the action of alkaline proteinases in the muscles increases. As a result, in myopathic muscles the relative content of MyHC IIb isoform decreases, the relative content of MyHC IIa and IId increases and the twitch characteristics of the skeletal muscle change.

SIMILARITY BETWEEN EXERCISE AND CORTICOSTEROID MYOPATHIES

It seems that there is some similarity between the structural changes in F-G and F-OG muscle fibres in case of exercise myopathy, although destructive changes in the G muscle fibres seem to be of smaller scope. On the one hand, this can be explained by the fact that G fibres do not participate actively in the low-intensity exercise. On the other hand, the large range of destructive processes in these fibres shows that G muscle fibres participate in long-term low-intensity exercise, performing the junction of the skeletal muscle in the role of an organ.

It is well known, that FT muscle fibres are more sensitive to the action of corticosteroids. Therefore it is probable that the increase of endogenous corticosterone level in endurance-type exhausted rats and cortisol level in overtrained athletes may be the important factor in the pathogenesis of the exercise myopathy.

Similarity between exercise and corticosteroid myopathy on the muscle fibre level consists in the following ST muscle fibres which have relatively high oxidative potential are much less sensitive to the destruction of myofibrils. FT muscle fibres are more sensitive to the catabolic action on the level of myofibrils. In case of exercise myopathy the OG muscle fibres are more damaged and in case of corticosteroid myopathy G fibres. Similarity on the skeletal muscle as the organ level is that in both cases the PWC and muscle strength is decreasing.

In case of exercise myopathy, in FT skeletal muscles with relatively high oxidative potential the turnover rate of MyHC is decreasing.

In case of corticosteroid myopathy the turnover of MyHC is decreasing in FT musles with low oxidative potential.

In conclusion: although there are similarities between development of exercise and corticosteroid myopathy there is no ground to say that exercise myopathy is the mild form of corticosteroid myopathy.

CONCLUSIONS

1. Destruction of myofibrils in volume-overtrained and glucocorticoid caused myopathic rats begins with disarray of thick myofilaments and spreads over the contractile apparatus in fast-twitch muscles. In case of overtraining destructions mainly appeared in oxydative-glycolytic fibres and in case of glucocorticoid myoptahy in glycolytic fibres. In both cases the degradation of contractile proteins is increasing.
2. In volume-overtrained rats the relative content of myosin heavy chain isoforms I and IIb decrease in fast-twitch muscle. The relative content of myosin heavy chain IId isoform is increasing in fast-twitch muscles with higher oxidative potential.
3. In glucocorticoid caused myopathic rats myosin heavy chain IIb isoform relative content is decreasing and myosin heavy chain IId isoform is increasing in fast-twitch muscles and glycolytic fibres. Myosin heavy chain IIa relative content is increasing in fast-twitch muscles.
4. Myosin heavy chain I and IIa isoforms are synthesized faster than myosin heavy chain IIb isoform. Myosin heavy chain IIb isoform has the highest sensitivity to the alkaline proteinase and the sensitivity is higher in fast-twitch muscles with lower oxidative potential.
5. The synthesis rate of myosin heavy chain, Troponin-T and protein C is decreasing with excessive increase of endurance training volume. The physical working capacity of experimental animals is decreasing with the decrease of myosin heavy chain and protein C synthesis rate and decrease of myosin heavy chain isoform I relative content in skeletal muscle.

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SUMMARY IN ESTONIAN

Müofibrillaarvalkude kompositsioon ja uuenemine skeletilihastes ületreenituse ja kortikosteroidse müopaatia puhul

Töö eesmärgiks oli uurida muutusi müofibrillaarsete valkude uuenemiskiiruses ja võrrelda müofibrillide destruktsiooni kiiretes ja aeglastes lihastes ning nende kiutüüpides ületreenituse ja kortikosteroidse müopaatia puhul.
Töös püstitati järgmised ülesanded:

Võrrelda müofibrillide destruktsiooni ja kontraktiilsete valkude degradatsiooni skeletilihases ületreeningu ja glükokortikoidse müopaatia puhul.

Uurida müosiini raskete ahelate sünteesi intensiivsuse ja suhtelise sisalduse muutusi kiiretes ja aeglastes skeletilihastes ning kiutüüpides ületreenituse ja glükokortikoidse müopaatia puhul. Samuti uurida müosiini raskete ahelate tundlikkust aluselise proteinaasi suhtes.

Leida seos vastupidavustreeningu mahu ja muutuste vahel üksikutes müofibrillaarsetes valkudes ning seos vastupidavustreeningu mahu ja kehalise töövõime vahel.

Töö tulemused näitavad, et kiire iseloomuga skeletilihastes algab müofibrillide destruktsioon nii ületreenituse kui ka kortikosteroidse müopaatia puhul müosiini filamentidest ja levib edasi üle kogu kontraktiilse aparaadi. Ületreenituse müopaatia puhul on kahjustatud põhiliselt oksüdatiiv-glükolüütilised lihaskiud, kortikosteroidse müopaatia puhul aga glükolüütilised lihaskiud. Mõlemal juhul on iselomulik intensiivistunud kontraktiilsete valkude degradatsioon.

Kiiretes skeletilihastes väheneb ületreenituse müopaatia puhul müosiini raskete ahelate I ja IIb isovormi suhteline sisaldus. Müosiini raskete ahelate IId isovormi suhteline sisaldus suureneb nendes kiiretes lihastes, kus oksüdatiivne potentsiaal on kõrgem. Kiiretes lihastes ja glükolüütilistes lihaskiududes langeb glükokortikoidse müopaatia puhul müosiini raskete ahelate IIb isovormi suhteline sisaldus ning suureneb IId isovormi suhteline sisaldus. Samuti suureneb kiiretes lihastes müosiini raskete ahelate IIa isovormi suhteline sisaldus. Müosiini raskete ahelate isovormide sünteesi intensiivsuse võrdlus näitas, et I ja IIa isovormi süntees on oluliselt kiirem kui IIb isovormi süntees. Müosiini raskete ahelate IIb isovorm on kõige tundlikum aluselise proteinaasi suhtes, eriti nendes kiiretes lihastes, kus oksüdatiivne potentsiaal on madal. Ülemäärase treeningumahu tõusuga kaasneb müosiini rakete ahelate, troponiin-T ja müosiini siduva C-valgu sünteesi langus. Kehalise töövõime langusega kaasneb skeletilihastes müosiini raskete ahelate ja müosiini siduva C-valgu sünteesi intensiivsuse langus ning müosiini raskete ahelate I isovormi suhtelise sisalduse langus.

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PUBLICATIONS

CURRICULUM VITAE

Priit Kaasik

Born 3 August 1967 in Tartu, Estonia
e-mail: priit.kaasik@ut.ee

Education

1974–1985	Tartu 3th Secondary School
1985–1991	University of Tartu, Faculty of Exercise and Sport Sciences
1992	MSc"Changes in the contractile apparatus of skeletal muscle after speed and strength training; possibilities of their evaluation"
1997–2002	PhD student at the Institute of Exercise Biology

Professional Emploiment

1991–1992	University of Tartu, Lab of Kinesiology, researcher
1992–2000	University of Tartu, Institute of Exercise Biology, researcher
2000–	University of Tartu, Institute of Exercise Biology and Physiotheraphy, lecturer

Specialized Courses

1993	Int Symp "Skeletal Muscle Research" Part I at University of Kuopio
1994	Int Symp "Skeletal Muscle Research" Part II at University of Kuopio, Depart of Physiology
1995	Int Symp "Skeletal Muscle Research" Part III at University of Jyväskylä
1996	CIMO grant to study at University of Jyväskylä
1997	University of Jyväskylä
1999	University of Copenhagen

Membership in professional organizations:

member of the Baltic Laboratory Animals Science Association
member of the Estonian Physiological Society
member of the Estonian Olympic Academy

Scientific activity

Research field: hormonal myopathies, hypertrophy and atrophy of skeletal muscle, morphology of skeletal muscle, the synthesis and degradation of the contractile and regulatory proteins in skeletal muscle

The total number of scientific publications is 72, including 7 articles in international peer-reviewed journals.

ELULOOKIRJELDUS

Priit Kaasik

Sündinud 3. augustil 1967 Tartus, Eestis
e-mail: priit.kaasik@ut.ee

Haridus

1974–1985	Tartu 3. Keskkool
1985–1991	Tartu Ülikool, kehakultuuriteaduskond
1992	sporditeaduste magister, dissertatsiooni teema "Kiirus- ja jõutreeningu mõjul tekkinud muutused skeletilihaste kontraktiilses aparaadis ja nende hindamise võimalused"
1997–2002	doktorantuur Tartu Ülikooli spordibioloogia instituudi, funktsionaalse morfoloogia õppetoolis

Erialane teenistuskäik

1991–1992	Tartu Ülikool kehakultuuriteaduskond, kinesioloogia labor, teadur
1992–2000	Tartu Ülikool kehakultuuriteaduskond, spordibioloogia instituut, funktsionaalse morfoloogia õppetool, teadur
2000–	Tartu Ülikool kehakultuuriteaduskond, spordibioloogia ja füsioteraapia instituut, lektor

Erialane enesetäiendus

1993	kursused teaduritele ja doktorantidele "Skeletal Muscle Research" Part I Kuopio Ülikoolis;
1994	kursused "Skeletal Muscle Research" Part II Kuopio Ülikoolis
1995	kursused "Skeletal Muscle Research" Part III Jyväskylä Ülikoolis
1996	CIMO grant tööks Jyväskylä Ülikooli Terviseteaduste ja liikumisravi osakonnas.
1997	metoodiline täiendamine Jyväskylä Ülikooli Terviseteaduste ja liikumisravi osakonnas (immuunohistokeemia, immuunobloting).
1999	Kopenhageni Ülikool

Erialaroganisatsioonid

Eesti Füsioloogia Seltsi liige
Eesti Olümpiaakadeemia liige
Balti Laboratoorsete Katseloomade Seltsi liige

Teadustegevus

Peamised uurimisvaldkonnad: hormonaalsed müopaatiad, skeletilihaste hüpertroofia ja atroofia, skeletilihaste kontraktiilsete ja regulatoorsete valkude ning nende isovormide süntees ja degradatsioon.

Kokku on ilmunud 72 teaduspublikatsiooni, sh 7 artiklit rahvusvahelistes eelrefereeritavates ajakirjades ja kogumikes.

DISSERTATIONES KINESIOLOGIAE UNIVERSITATIS TARTUENSIS

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