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6

EFFECT OF MECHANICAL LOADING AND AGEING ON MYOSIN HEAVY CHAIN TURNOVER RATE IN FAST-TWITCH SKELETAL MUSCLE

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LIST OF ORIGINAL PUBLICATIONS

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- I. **Pehme A., Alev K., Kaasik P., Julkunen A., Seene T.** The effect of mechanical loading on the MyHC synthesis rate and composition in rat plantaris muscle. Int. J. of Sports Medicine (accepted 2003, in press).
- II. Pehme A., Alev K., Kaasik P., Seene T. Age related changes in skeletal muscle myosin heavy chain composition: effect of mechanical loading. Journal of Ageing and Physical Activity, 2004, 11: 29–44.
- III. Umnova M., Pehme A., Seene T., Mitashov V., Tjutchkova S. Response of rat skeletal muscle fibers and neuromuscular junctions of strength exercise training (electron microscopic and radioautographic study). Dokladô Akademij Nauk, 1998, 359, 5: 702–706 (in Russian).
- IV. Umnova M., Seene T., Krasnov I., Pehme A. A study of ultrastructural characteristics of rat neuromuscular junctions under physical loads. Cytology, 2000, 42, 10: 983–92. (in Russian).
- V. Pehme A., Seene T. A model for strength exercise of skeletal muscles in the rat. Scand. J. Lab. Anim. SCI. 1996, 23, (Suppl.1): 419–424.

ABBREVIATIONS

ATP	_	adenosine triphosphate
b.w.	_	body weight
BSA	_	bovine serum albumin
СН	_	compensatory hypertrophy
DNA	_	deoxyribonucleic acid
ECL	_	enhanced chemiluminesence
EDTA	_	ethylendiaminetetraacetic acid
FGF	_	fibroblast growth factors
HPLC	_	high-pressure liquid chromatography
IGF-1	_	insulin like growth factor-1
MGF	_	mechanical growth factor
mRNA	_	messenger ribonucleic acid
MyHC	_	myosin heavy chain isoform
MyLC	_	myosin light chain isoform
SDS-PAGE	_	sodium dodecylsulphate polyacrylamide gel electrophoresis
PVDF	_	polyvinyldene difluoride transfer
RSA	_	relative specific activity
RT	_	resistance training
SA	-	specific activity
TGF	_	transforming growth factors
3-MeHis	_	3-Methylhistidine

INTRODUCTION

When using high-intensity and low-repetitive exercise, skeletal muscles show marked gains in strength that are due both to neuronal adaptations and to an increase in the cross-sectional area of muscle. There is consensus that the gain in the cross-sectional area of muscle is mainly due to an increase in myofibrillar proteins. The cross-sectional area of all fibre types is increases after resistance training, with a tendency to larger increases in type II than type I fibres (Hortobagyi *et al*, 2000). Volume densities of mitochondria are found to be reduced and may be as low as a few per cent in some elite athletes due to dilution of the constant mitochondrial volume in larger muscle fibres.

It is still not fully known whether specific types of mechanical loading can induce specific types of adaptations or whether the muscle simply responds in a stereotypic fashion to any increase in the mechanical load. Several animal models have been used to induce and study the mechanisms of muscle enlargement like tenotomy of synergistic muscles, ablation of synergistic muscles, passive stretch, and numerous exercise-induced models (Timson, 1990; Noirez *et al*, 2000).

Compensatory hypertrophy is characterized by an increase in muscle mass, muscle protein, contractile force, and by a shift from the fast to slow myosin type in fast-twitch muscle (Timson, 1990). Contractile activity can induce differential expression of myosin protein isoforms in skeletal muscle. Many different types of studies of the striated muscle focus on the assessment of the composition of the myosin heavy chain (MyHC), because of its important regulatory role in myosin ATPase activity and, therefore, velocity of muscle fibre shortening (Bottinelli, 2001; Gür et al, 2003). MyHC has muscle fibre type specificity and is encoded by the multigene family, which is mapped to a single chromosome (Carson et al, 2002; Flück & Hoppler, 2003). The modulation of MyHC protein in adult skeletal muscle is multifactoral, as many factors like developmental, neural, hormonal, and mechanical participate in this process (Baldwin et al, 1990; Bottinelli, 2001; Flück & Hoppler, 2003). It has been demonstrated that mechanical loading is more responsible for the modulation of MyHC isoform expression than stimulation frequency (Caiozzo et al, 1997). Pre-translational mechanisms of MyHC protein regulation are highly sensitive to even small amounts of resistance training (Caiozzo et al, 1996). As the protein remodelling process consists of protein breakdown and synthesis, it is important to know the synthesis rate of MyHC isoforms in oder to better understand mechanisms of muscle hypertrophy. Previous reports have shown that different types of mechanical activity affect the synthesis rate of MyHC and the myosin light chain (MyLC) in the skeletal muscle (Seene & Aley, 1991). The reports concentrate mostly on the relative content of isomyosins or MyHC isoforms under different mechanical factors and types of activities (Campos et

al, 2002; Frederiksen & Christensen, 2003). However, only few researchers pay attention to the synthesis of MyHC isoforms in skeletal muscle (Balagopal *et al*, 2001; Hasten *et al*, 2000). Although some results suggest that mechanical factors have an important role in controlling the expression of contractile proteins (Caiozzo *et al*, 1997), the influence of the quantity and type of mechanical loading on old muscle is still unknown. It has been suggested that changes in muscle structure, mass, MyHC concentration, and its isoform expression induced by a high-resistance weight-training programme are a result of the frequency of contraction (Deschenes *et al*, 2000; Hunter *et al*, 2001, Tikunov *et al*, 2001). There is no explicit understanding of how synthesis intensity, composition of MyHC isoforms, and the MyHC turnover rate change with age. Nor do we know the limits of protein synthesis and adaptative capacity in the case of different mechanical loading factors.

It seems unrealistic that all type of resistance exercise should lead to a decrease in the fastest MyHC isoform in skeletal muscle (MyHC IIb in small laboratory animals and MyHC IIx in human). There is some ground to believe that above-mentioned changes in MyHC isoforms a result from used proportions of resistance training power (intensity) and volume. It has been shown that the MyHC IIb isoform is highly sensitive to the action of proteinases (Seene *et al*, 2003). It has also been shown that the MyHC IIb isoform is sensitive to an increase in exercise training volume (Demirel *et al*, 1999). The present study used the effect of resistance training programmes with different ratios of power and volume. The reaction of the relative content MyHC isoforms and synthesis rate was characterized in detail in mature and ageing fast-twitch skeletal muscle.

REVIEW OF LITERATURE

1. SKELETAL MUSCLE FIBRE TYPES ACCORDING TO MyHC ISOFORMS

Skeletal muscle fibres exhibit a high degree of spezialization. Muscle fibres with low oxidative potential (glycolytic fibres), generate force rapidly, but fire after brief activity, reflecting the prevalence of fast-myosin isoforms. Skeletal muscle fibres with a high oxidative potential can express slow-myosin isoforms in addition to fast-myosin isoforms; they are rich in mitochondria and resist fatigue. MyHC isoforms in single fibre fragments have led to the delineation of pure and hybrid fibres (Pette & Staron, 1990). Pure fibre types, for example, type IIB, type IID/X, type IIA, and type I, express MyHC IIb, MyHC IId/x, MyHC IIa, and MyHC I β , respectively, whereas hybrid fibres express more than one MyHC isoform. The percentage of hybrid fibres increases remarkably in transforming muscles, for example, up to 60% in fast-to-slow transforming rabbit muscle (Pette, 2001).

Time course studies on fast-twitch muscles of the rat and the rabbit show that fast-to-slow conversion encompasses sequential MyHC isoform exchanges in the direction of MyHC IIb to MyHC IId/x to MyHC IIa to MyHC I β , corresponding to fibre-type transitions from type IIB to type IID/X to type IIA to type I. This is complemented by hybrid fibres, which, according to their coexisting MyHC isoform patterns (MyHC IIb + MyHC IId/x, MyHC IId/x + MyHC IIa, MyHC IIa + MyHC I β) bridge the gaps between the pure fibre types (Conjard *et al*, 1998).

It is possible to combine the data from fast-to-slow and slow-to-fast transforming muscles in a general scheme of reversible transitions in MyHC isoform expression, namely, MyHC IIb \leftrightarrow MyHC IId/x \leftrightarrow MyHC IIa \leftrightarrow MyHC I β (Pette & Staron, 2000). According to this scheme, fibre-type transitions occur in stepwise manner, econompassing up- and down-regulations of MyHC isoforms in a gradual sequence. Depending on their position in the MyHC isoform spectrum, some fibres have the ability to transform in either direction. Fiber-type-specific options for transformation in the fast or slow direction could explain species-specific (Jaschinski *et al*, 1998) and muscle-specific differences in response to altered functional demands.

2. EFFECT OF COMPENSATORY HYPERTROPHY ON SKELETAL MUSCLE

When surgically removing particular muscle synergists, all fibres enlarge in response to the mechanical overload. In addition to hypertrophy, MyHC I isoform becomes the preferential type expressed (Roy et al, 1985). So, the continuous mechanical application induces a transformation across the chronically overloaded muscle fibre pool by expanding the relative size and number of slow fibres to better enable the muscle to withstand the chronic overload while retaining functional properties in another pool of fibres that remain more genetically programmed for performing maximal intensity type of activity. Transformation in contractile protein expansion and contractile protein isoform expression is mediated through transcriptional/pre-translational and translational processes because the level of mRNA for the specific MyHC isoforms is alters along with the changes in protein accumulation (Swoap *et al*, 1994). In comparison with heavy-resistance training, where the majority of fibres hypertrophy in response to the stimulus, MyHC IIb isoform is downregulated and IIa and IId/x are up-regulated (Haddad *et al.*, 1998). It seems that the transformation in MyHC protein phenotype in response to mechanical stress is highly specific to both the level of force and duration. Furthermore, the changes in the MyHC phenotype seem to be heavily influenced by pretranslational processes due to the parallel shifts in both mRNA and protein content that occur for a given MyHC isoform (Baldwin & Haddad, 2002). Muscle adaptation in response to resistance training does not involve appreciable up-regulation of the mitochondrial system, which further illustrates the specificity of the adaptive stimulus.

3. EFFECT OF RESISTANCE TRAINING ON SKELETAL MUSCLE

The strength and oxidative capacity of skeletal muscle are major determinants of physical performance. Exercise training augments the functional capacity of skeletal muscle by altering the abundance of proteins essential for contraction and energy metabolism. Adaptation results from exercise-induced changes in the abundance of specific mRNA transcripts. It shows that gene transcription is an important target for signalling pathways that couple contractile activity to changes in muscle phenotype.

Resistance exercise did not cause adaptation not only on the skeletal muscle fibre level, but also on the level of neuromuscular junctions. Endplate perimeter length and area increased, and the dispersion of acetylcholine receptors within the endplate region enhanced (Deschenes *et al*, 2000).

Adaptational changes in the skeletal muscle to resistance training make it less endurable. It seems that the skeletal muscle becomes less economical as force production capacity and maximal isometric strength increases (Jensen *et al*, 1997). Decrease in metabolic economy may be related to increased dependence on inefficient type II muscle fibres (Hunter *et el*, 2001).

3.1. Fibre Type Transformation

Resistance training is a potent stimulus with dramatic effects on muscle phenotype.

Adaptation of fast-twitch muscle fibres to resistance training exhibits a transition to a large proportion of MyHC oxidative isoforms (MyHC I and IIa). Recent data indicate that the increase in pure type MyHC IIa expressing fibres can be attributed to a decrease in the number of fibres that are hybrid with regard to the expression of slow and fast MyHC isoforms (Williamson *et al*, 2001). The eventual drop in total MyHC IIx expression as a consequence of differentiation of MyHC IIa-expressing hybrid fibres towards pure MyHC IIa-expressing fibres. The increase in the size of pure MyHC IIx-expressing fibres seems to be of less importance.

If subjects are detrained for several weeks, it appears that this process is reversed and the re-expression of the fast type MyHC IIx in (hybrid) fibres appears to be greater than in the pre-training period (Andersen & Aagaard, 2000). However, even though resistance training increases type IIa MyHC expression, it is uncertain how these molecular changes relate to the functional changes seen with strength training, in particular at the initial stages of strength training (Carroll *et al*, 1998).

4. EFFECT OF CHARACTER OF RESISTANCE TRAINING ON THE SYNTHESIS RATE OF MUSCLE PROTEINS

For any protein to undergo altered expression, a highly specific adaptive stimulus must be applied to the cell system of sufficient intensity, duration, and frequency (Baldwin & Haddad, 2002). Typical heavy resistance training is relatively short in the form of a series of individual contractions. At present we do not fully understand the optimal conditions, necessary for inducing adaptations in most cellular systems during resistance training. There should be a critical threshold stimulus needed to induce an adaptation in MyHC expression and muscle fibre hypertrophy. It has been shown that approximately 50 near maximal high resistance contractions, performed in a single training

session, are sufficient to transiently induce both the level of mRNA and the rate of protein synthesis for marker contractile proteins (Baldwin & Haddad, 2001). If the stimulus is repeated a sufficient number of times (12–15 training session) at sufficiently close intervals as a minimum of every other day, the collective process to induce a net expansion of the contractile protein pool could be insufficient to result in a measurable degree of muscle fibre enlargement.

4.1. Effect of Resistance Training on the Synthesis Rate of Myosin Heavy Chains

Previous studies have shown that different types of mechanical activity affect the synthesis rate of MyHC in skeletal muscle (Seene & Alev, 1991). The effect of resistance training on the MyHC synthesis rate have been studied mostly indirectly according to changes in the relative content of its isoforms. Only few studies pay attention on the synthesis of MyHC isoforms (Hasten *et al*, 2000; Balagopal *et al*, 2001). In comparison with other main contractile protein actin, the MyHC isoforms synthesis rate is more sensitive to the hormonal influences particularly in fibre with a have low oxidative potential (Seene *et al*, 2003).

4.2. Effect of Resistance Training on the Turnover Rate of Myofibrillar Proteins

All proteins in mammalian muscle fibres are in a continous process of being synthesized and subsequently degraded. The balance between protein synthesis and degradation determines whether there is either hypertrophy or atrophy in muscle mass. This fundamental process also allows qualitative remodelling of the muscle so that one isoform, for example, is replaced by another that is better suited for specific conditions (Baldwin & Haddad, 2002). Further, the inherent turnover rate of a given protein is dictated by its half-life. Muscle myofibrillar proteins have relatively long half-lives (Baldwin & Haddad, 2002). The half-life of myofibrillar proteins is significantly reduced if these proteins are disassembled, as seen during low contractile activity.

Therefore, the process of protein turnover provides a mechanism in which both the type and amount of protein comprising cellular systems can be adapted in accordance with the environmental conditions imposed.

Experiments point to the importance of changes in protein turnover for the hypertrophic process (Wong & Booth, 1990). Resitance training is known to increase the fractional synthesis rate and the fractional breakdown rate within 3 h after exercise that are maintained up to 48 h after the training in humans (Phillips *et al*, 1999). Mixed protein and MyHC synthesis rates remain unchanged as a consequence of continued resistance training of young and

elderly subjects (Hasten *et al*, 2000; Balagopal *et al*, 2001). Due the lack of pertinent expression data, it is currently not known whether the absence of mitochondrial adaptations with strength training is due to transcriptional regulatory events or enhanced mitochondrial turnover.

5. EFFECT OF RESISTANCE TRAINING ON THE RELATIVE CONTENT OF MyHC ISOFORMS

Resistance exercise training expands the amount of the myofibrillar apparatus in order to enlarge fibre cross-sectional area (Baldwin & Haddad, 2002). Also, there is a concomitant alteration in contractile protein phenotype and metabolic enzyme levels, which seems to occur in accordance with activity-induced changes in the muscle's fibre-type profile.

It has been shown that the repetition regime in the resistance training protocol plays an very important role in the hypertrophy of muscle fibres. High repetitions in resistance training did not cause any significant hypertrophy of muscle fibres (Campos *et al*, 2002).

Resistance training in small animals causes a decrease in the percentage of type IIB fibres with a concomitant increase in IIAB fibres. Fibre type conversion was supported by a significant decrease in MyHC IIa isoform (Campos *et al*, 2002).

Heavy-load resistance training in human decreased the amount of MyHC IIx isoform and reciprocally increased the MyHC IIa content. Detraining following heavy-load resistance training seems to evoke an overshoot in the amount of MyHC IIx isoform to markedly higher values than those observed prior to resistance training (Andersen & Aagaard, 2000).

6. DEVELOPMENT MECHANISM OF MUSCLE FIBRE HYPERTROPHY

Animal models have clearly shown that satellite cell activation is involved and may be a prerequisite for fibre hypertrophy (Schultz & McCormick, 1994). Using a marker for satellite cells, it has been documented that expression of early markers of myogenesis is activated in satellite cells and muscle fibres in response to resistance training in humans (Kadi & Thornell, 1999). Satellite cells are believed to proliferate and fuse with the existing fibres, thereby contributing to an increase in myonuclei per muscle fibre. The number of myonuclei increased with fibre hypertrophy and positively correlated with the increased number of satellite cells (Kadi & Thornell, 2000). This suggests that additional myonuclei are needed to support the enlargement of muscle cells during strength training. Moreover, hypertrophy of the skeletal muscle of untrained elderly men induced by high-intensity resistance training did not result in significant changes in the cytoplasma-to-myonucleus ratio despite the increased cross-sectional size of all fibre types and transition of type IIX towards IIA fibres (Hikida *et al*, 2000). The cellular changes occurring during hypertrophic adaptations essentially confirm the nuclear domain theory, suggesting that the cytoplasma-to-myonucleus ratio is a function of the myosin type and the amount of protein turnover (Booth & Baldwin, 1995). It seems that satellite cells are recruited during hypertrophy of muscle fibres in order to maintain the cytoplasma-to myonucleus ratio.

It was shown that in the trained subjects who showed muscle fibre hypertrophy, there was a significant increase in the number of fibres that stained for embryonic and fetal myosin heavy chains, normally not expressed in adult muscle fibres (Kadi & Thornell, 1999). These findings show an alternative way for forming new fibres. If the number of newly formed fibres exceeds the number lost by damage, this will lead to an increase in the number of fibres (Kadi &Thornell, 2000).

Thornell *et al* (2003) have observed that the satellite cell number is increased in top-level powerlifters. This increased number of satellite cells could be due to asymmetric division of satellite cells and will make muscle more responsive to further adaptation (Kadi & Thornell, 2000; Vierck *et al*, 2000). The number of satellite cells in skeletal muscle decreases with age (Thornell *et al*, 2003). The expression of mRNAs for transforming growth factors (TGF- β 2; myostatin, actinin- β , and follistatin), IGF I and II, and fibroblast growth factors (basic, bFGF) was investigated in satellite cells in the stages from initiation of proliferation to fusion (Kocamis *et al*, 2001).

6.1. Role of Growth Factors in Muscle Hypertrophy

Recent gene profiling data show that work-induced muscle hypertrophy is an integrated transcriptional response whereby genes related to carbohydrate and protein metabolism, autocrine/paracrine factors, extracellular matrix proteins, transcription factors, and cell-regulatory factors change together (Carson *et al*, 2002). An increase in the muscle-regulatory factor myogenin in overloaded muscle supports its involvement as a major controller of the complex fast-to-slow transformation process (Carson *et al*, 2002). Another influence in muscle hypertrophy seems to be the ski gene (Sutrave *et al*, 1990). It has been demonstrated that type II fast fibres undergo selective hypertrophy in muscles of transgenic mice that overexpress the ski gene. Clear evidence for the role of the ski gene in muscle hypertrophy comes from experiments indicating a threefold increase in ski mRNA after injury (Soeta *et al*, 2001). mRNA has been

identified as an additional factor of potential importance for muscle hypertrophy (Hespel *et al*, 2001). It has been demonstrated that a single bout of resistance exercise in young male subjects has significant effects on the transcriptome (Jozsi *et al*, 2000).

Peptide growth factors control the molecular steps by which multipotent progenitor cells differentiate into skeletal myocytes. Insuline growth factors (IGF-I) stimulate differentiation in satellite cells. It has been shown that IGF-I is involved in the hypertrophy of skeletal muscle fibres (Harridge, 2003). Muscle cell culture studies have clearly demonstrated that IGF-I has an anabolic function as shown by its ability to increase the diameter of myotubes, suppress protein degradation, increase amino acid uptake, and stimulate protein synthesis (Rommel et al. 2001). In addition to hepatic IGF-I there is local synthesis of IGF-I in other tissues, including muscle. The local production of IGF-I suggests that it has important autocrine/paracine functions. IGF-I released into the surrounding tissue/circulation may bind to one of a number of binding proteins. The binding proteins serve to stabilize and transport IGFs from the cirulation to peripheral tissues, maintain a reservoir of IGFs in the interstitial tissue/ irculation, potentiate or inhibit IGF action as well as mediate IGF-independen biological effects. IGF-IEc isoform was sensitive to mechanical signals or the micro damage caused and prompted this isoform to be termed mechano-growth factor or MGF (Yang et al, 1996). When stretch was combined with electrical stimulation of the tibialis muscle MGF mRNA up-regulation was greater than that caused by stretch alone (Mckov et al. 1999). Secondly, the mRNA for MGF could not be detected after stretch and immobilization in MDX mice (Goldspink et al. 1996), those transgenic animals who do not contain the gene for the important structural cytoskeletal protein dystrophin. Furthermore, recent studies by Haddad and Adams (2001) showed that the mRNA for MGF was elevated by at least tuofold after a single bout of isometric exercise in rats.

7. AGEING AND MUSCLE STRENGTH

The ageing process is associated with a number of physiological changes. One of the most marked of them is the loss of muscle mass or sarcopenia. Muscle has obvious mechanical functions, but it also serves as a dynamic metabolic store, a source of heat, and a form of protective padding, all of which are negatively effected by sarcopenia (Griffiths *et al*, 2001). With increasing age, muscle strength may eventually decline to a level where weakness starts to restrict the ability to carry out everyday tasks. Cross-sectional studies have demonstrated that muscle strength is significantly, however, not linearly, associated with functional limitations. Poor muscle strength has been found to be associated with older age (Rantanen, 2003).

Beginning in midlife, ageing is associated with a time-dependent loss of muscle mass. It is a major cause of disability, frailty, and loss of independence in the mainly due to the associated loss of muscle strength and to a lesser extent stamina (Dorres & Rennie, 2003).

Decrease in muscle strength is associated with a decrease in the crosssectional area of the muscle fibres and in capillary bed density as well as in a reduction in the number of muscle fibres (Frontera *et al*, 2000). These morphological and metabolic modifications in the skeletal muscle can be explained in part by reduced physical activity, altered nutritional status, and disease factors, which accompany the ageing process (Roubenhoff & Hughes, 2000). In addition, some recent results indicate that functional/structural properties of contractile proteins and in particular the slow MyHC isoform is altered upon ageing leading to a slowdown of the contractile speed (Hook *et al*, 2001). Strategies to prevent or reduce sarcopenia receive increased attention due to pressures on health care by growing numbers of elderly members of society. Unfortunately, the links between causes and effects are not easy to discern. Wasting is associated with a loss of tissue protein, and this must mean an imbalance between the rates of tissue protein synthesis and breakdown.

7.1. Age-Related Changes in Motor Units

All muscles are governed by motor units, that is a neuron and the fibres innervated by the nerve, which on the basis of their physiological characteristics are separated into slow and fast-twitch units with subtypes. The muscle fibres of slow motor units are termed type I fibers, contain slow-twitch MyHC, contract slowly contracting and resist fatigue. Human fast motor units are composed of type II fibres. They contain different fast isoforms of MyHC, contract fast, show, depending on the subtype, various degrees of resistance to fatigue, and behave differently upon ageing (Dorres & Rennie, 2003). It is well known that motor units are lost as a result of ageing. The motor unit remodelling process expresses itself by changing of type I and II fibres into one in which fibres of the same type are grouped together (Dorres & Rennie, 2003; Harridge, 2003).

It is, in fact, likely that sarcopenia is caused by a combination of many factors (Volpi *et al*, 2001).

Loss of muscle mass is mainly caused by loss of type II fibres and a reduction of fibre size, beginning at the age of ~ 25 years and accelerating thereafter. By the age of 50 years approximately 10% of the muscle area is lost, and the average reduction in muscle area in *vastus lateralis* between 20 and 80 years is 40% (Lexell, 1995). The reduction of fibre size is due to selective atrophy of fast-twitch muscle fibres; slow-twitch fibers are less affected.

8. CHANGES IN MUSCLE PROTEIN SYNTHESIS IN OLD AGE

The reasons why muscle fibres are lost and fast-twitch fibres atrophy, and protein synthesis rate decreases in old age remain important questions in the aethiology of sarcopenia (Harridge, 2003).

There are many discrepancies in the extent of changes and in the rates of protein synthesis in multiple muscle fractions. Hasten *et al* (2000) found the synthetic rate of MyHC to be 40% lower in elderly subjects, but when they measured mixed muscle protein and actin synthetis rates, there were no significant differences. Balagopal *et al* (1997) reported lower rates of MyHC, and mixed muscle protein synthesis rates were similar in the young and the elderly.

8.1. Effect of Resistance Training on Muscle Protein Synthesis

It is agreed that resistance exercise stimulates muscle protein synthesis in both the young and the elderly. There was a 30% increase after a single bout of exercise between young trained subjects and untrained controls (Chesley *et al*, 1992). The fractional rate of protein synthesis in both young and old subjects increased 36% and 60%, respectively, after two weeks of resistance exercise (Yarasheski *et al*, 1993). There is conflicting data about protein synthesis during exercise training Yarasheski *et al* (1999), Hasten *et al* (2000), Balagopal *et al* (2001) all report that synthesis rates of MyHC and mixed muscle proteins did increase with exercise whereas Welle *et al* (1995) found that there was no significant increase in myofibrillar protein synthesis. The nature of the effect is still unclear.

In untrained subjects a single exercise session increased mean muscle protein synthesis 112% after 3 h, 65% after 24 h, and 34% after 48 h (Phillips *et al*, 1999). However, in trained subjects the values for protein synthesis are much lower: 50% 4 h after a single bout of resistance exercise and only 14% after 36 h. Even short-term weightlifting exercise increases the synthesis rate of contractile proteins both in young and ageing persons MyHC and mixed protein. MyHC and mixed protein synthesis rates are reduced more than actin synthesis rate in advanced age (Hasten *et al*, 2000). The magnitude of the effect on muscle protein synthesis probably depends on the relative intensity of the exercise and amount of training the subject is accustomed to. However, because repeated strenuous resistance exercise is anabolic, there is no reason to suspect a higher basal rate of synthesis.

8.2. Disuse Atrophy in Elderly Muscle

There were no strength training induced increases in strength in 20–30-year-old men and women (34%) compared to 65–75-year-old men and women (28%) (Lemmer *et al*, 2000). However, after 31 weeks of abstention from exercise there was a greater loss of strength in older than in young subjects. In agreement with this Ivey *et al* (2000) found that ageing in human subjects has no effect on the response of muscle to strength training although their results showed that there was no difference in detraining after 31 weeks either. These findings suggest that disuse atrophy does not entirely explain the decreases in muscular strength with advancing age and shows that elderly muscle responds to the same extent as young muscle to an intense training stimulus.

Physical inactivity is likely to contribute to sarcopenia. Even those individuals who are physically active throughout their lives demonstrate decreases in functional ability and appear to demonstrate losses in muscle mass (Roth *et al*, 2000).

9. UNSOLVED PROBLEMS

It is still not fully known how skeletal muscle responds to increase in mechanical load. It is known that compensatory hypertrophy is characterized by an increase in muscle mass, muscle protein, and contractile force and by a shift from the fast-to-slow myosin type in fast-twitch muscles, but the exact development mechanism of muscle hypertrophy is still open. MyHC is encoded by a multigene family, which is mapped to a single chromosome (Carson *et al*, 2002; Flück & Hoppler, 2003), but the mechanism of changing its isoform during resistance training is poorly understood. It is known that the modulation of MyHC in adult skeletal muscle is multifactoral, and many factors participate in this process (Bottinelli, 2001). However, one needs to clarify how transcription and growth factors act in mature striated muscle.

Ageing is characterized by changes in skeletal-muscle mass. The agerelated decrease in muscle-protein synthesis rate is related to diminished ribosomal capacity. This might cause a differential capacity between young and old skeletal muscle to respond to stimuli that cause increased contractile-protein synthesis and growth rates during compensatory hypertrophy and resistance exercise training. It has been shown that compensatory hypertrophy (CH) is age-related in rat *plantaris* muscle after tenotomy of the *gastrocnemius* at an early stage. The age-related slowing of contractile capacity with CH has not been studied in ageing rats but only in developing rat skeletal muscle (Kandarian *et al*, 1992). Although some results suggest that mechanical factors have an important role in controlling the expression of contractile proteins (Diffee *et al*, 1993; Caiozzo *et al*, 1997), the influence of the quantity and type of mechanical loading on old muscle is still unknown. It has been suggested that changes in muscle structure, mass, MyHC concentration, and its isoform expression induced by a strength training programme are a result of frequency of contraction (Deschenes *et al*, 2000; Hunter *et al*, 2001; Tikunov *et al*, 2001). Large contractile forces provide cellular signals that up-regulate the expression of slower myosin isoforms in skeletal muscles (Demirel *et al*, 1999; Andersen & Aagaard, 2000; Pette, 2001). However, we still do not know much about the fastest MyHC isoforms IIb. We do not know how the synthesis intensity of muscle contractile proteins, MyHC-isoform composition, and MyHC turnover rate change with age, nor do we know the limits of protein synthesis and adaptive capacity in the case of different mechanical loading factors.

AIMS OF THE STUDY

The purpose of the present study was to investigate the response of contractile proteins synthesis rate and the magnitude of MyHC isoforms transformation in fast-twitch skeletal muscle to different modes of mechanical loading and age-related responses.

The specific aims were as follows:

- 1. To study the role of satellite cells in the development mechanism of induced by resistance exercise training fast-twitch skeletal muscle hypertrophy.
- 2. To investigate the effect of different modes of mechanical loading on the synthesis and turnover rate of contractile proteins, myosin heavy chain isoforms and age-related responses in fast-twitch skeletal muscle.
- 3. To establish relations between changes in the relative content of myosin heavy chain isoforms in response to different modes of mechanical loading, hindlimb grip strength, and the capacity to carry maximal weight.

MATERIALS AND METHODS

1. Animals

Male Wistar rats (National Laboratory Animal Centre, Kuopio, Finland) were used. Rats were randomly divided into groups: the control group, resistance exercise training groups, compensatory hypertrophy groups, and a combination of compensatory hypertrophy and resistance training groups. The animals studied were young adults (12 weeks old at the beginning of experiments), adults (17 weeks old in the beginning of experiments) and old animals (80 weeks old at the beginning of experiments). All animals were housed in identical environmental conditions in polycarbonate type III cages, at 21°C, two per cage at 12/12 hours light/dark period (lights on at 8 a.m.). The rats received the diet SDS-RM1 (c) 3/8, Witham, Essex, England and water *ad libitum*.

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 monitored by the Committee of Laboratory Animal Science, University of Tartu.

2. Surgical Procedure

The animals were anaesthetized by intraperitoneal injection of ketamine (Calypsol, Gedeon Richter A.O. Budapest, Hungary) 2.5 mg/100 g body weight (b.w.) and diazepam (Lab Renaudin, France) 2.5 mg/100 g b.w. Unilateral compensatory hypertrophy (CH) was induced in the *plantaris* muscle of the right hindlimb by the tenotomy of the *Achilles* tendon of the *gastrocnemius* muscle (Hanzlikova *et al*, 1975).

3. Mechanical Loading

The mechanical loading on skeletal muscle was induced by resistance exercise in vertical treadmill, surgical tenotomy of a synergist muscle, or a combination of synergistic tenotomy and resistance exercise. After surgery the rats, young (n=18), adult (n=20), and old animals (n=18), were returned to their cages and randomly divided into the sedentary and resistance exercise training group. The sedentary animals remained in their cages for 30 days for the evolution of CH. The time period for the evolution of CH was selected on the basis of the earlier reports (Collnick *et al*, 1981). The resistance training animals started to adapt to the vertical treadmill after a five day recovery period after surgery before commencing the exercise programme. RT programs started on the 9th day after the surgery, and the training period lasted for 22 days, including 19 sessions per period. The animals were trained on a vertical treadmill at a speed of 18 m/min at a 80° angle for a distance of 1.5 m during 5 sec for 6 days or 3 days a week during 4 weeks or 6 weeks. RT consisted of 2–5 runs per session (Monday – 2, Tuesday – 3, Wednesday – 4, Thursday – 5, Friday – 4, Saturday – 3), recovery time 1.5 min between runs, and the peak frequency was in the middle of the week. The animals carried a progressively heavier extra weight on tails secured with a belt and elastic tape.

During the training period the rats carried extra weights on the flexible-rod vertical treadmill. The extra weight for the animals during the first week of exercise was 85 and 100 g depending on the group, young and old, respectively, constituting 25% of the b.w. During the second week of exercise, the extra weight was 100 g (28% of the b.w.) and 200–350 g depending on the group during the last week. In adult (control, n=6) RT groups 1 (n=6), 2 (n=6), and 3 (n=6) the extra weight during the first week of exercise was 100 g, which was increased weekly by 50 g, the final extra weight being 350 g. Gr 1 was trained three days per week, Gr 2, 6 days per week during the six-week training period. Gr 3 was trained six days per week, during four weeks. The total work and power of exercise was calculated as described earlier (Pehme & Seene, 1996).

The vertical treadmill is made up of a running track with an uphill inclination. A plexiglas cage and an electronic motor are fastened to the metallic ground plate of a 55 cm long and 38 cm wide control desk. The running track resembles a down-moving ladder (length 1500 mm, width 300 mm) with stainless rungs (5 mm in diameter) with 8 mm distances between each. The moving speed is 0-30 m/min.

A 80 cm high plexiglas cage (30 cm long and 15 cm wide) is fastened to the ground plate so that the back side of the cage forms the running track. On the bottom of the cage, there is an electrical stimulator with a mild current for the stimulation of the rat to learn to climeb. On the front side there is a door for putting the rat on the running track. At the end of the running track above the stimulator plates there is a rolling brush (3 cm in diameter) for the security the of the rat's tail and the hindlimb moving in the same direction with the running track. For the vertical treadmill drive motor with power 120 W; max speed 2700 rpm is used. The speed of the vertical treadmill is measured by electromagnetic counter 24 V, 25 cps. The tilt angle is changed by a mechanical goniometer (0 – 30°). The hindlimb grip strength (N) was measured before and after the training period with Grip Strength Meter 0167-004L (Columbus Instruments).

4. Muscle Sample Preparation

At the end of the CH protocol or 24 hrs after the last training session, the animals were anaesthetized as described in the surgical procedures and sacrificed. The *m. plantaris* glycolytic and oxidative-glycolytic fibres samples were separated from *m. quadriceps femoris* and quickly removed, trimmed clean of visible fat and connective tissue, weighed, frozen, and stored in liquid nitrogen pending further processing or fixed for ultrastructural studies. In order to investigate the specific activity of muscle protein fractions, the single isotope method was used. For the administration of labelled amino acid the large dose technique was used. L-[4.5 ³H] leucine (170 Ci/mmol) was infused intraperitoneally of 1.0 ml for 2 h, 200 μ Ci per 100 g body weight before the collection of muscle samples. The incorporated radioactivity was measured in a liquid scintillation counter. ³H thymidine was infused intramuscularly 30 μ Ci per animal. After 48 h rats were anaesthetized and sacrificed; ultrathin sections were covered with photoemulsion and were exposed for 8 weeks.

4.1. 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).

5. Separation of Total Muscle Cell, Myofibrillar, and Sarcoplasmic Protein Fractions

The minced muscle samples were homogenized in a buffer containing: 50 mM KCl, 10 mM K₂HPO₄, 1 mM EGTA, 1mM MgCl₂, 1 mM dithiothreitol, at pH 7.0 and analysed as the total muscle cell protein fraction. For further purification the homogenates were centrifuged at 1,000 g for 10 min, and the supernatant was taken as a sarcoplasmic fraction. The crude myofibrillar fraction was rehomogenized in the same buffer with 0.1% triton – 100 and centrifuged at 1,000 g for 10 min. The total muscle cell protein and myofibrils were dissolved in 0.3 M NaOH and analysed for radioactivity and protein, the ratio of which is specific activity (SA).

6. Separation of MyHC Protein

Muscle samples of the *m. plantaris* were pulverized under liquid nitrogen and homogenized in 5 vol water. The homogenate was mixed with an equal volume of 12% sodium dodecylsulphate (SDS) solution (1:1), and 2-mercaptoethanol 2% (vol/vol) was added. After incubation for 90 min at 60 °C with shaking, the mixture was centrifuged for 30 min at 3,000 g to remove the fat and unsolved connective tissue (Schreurs *et al*, 1983).

10% SDS-PAGE in tubes was used for separation and purification of the total MyHC protein (Porzio & Pearson, 1977). For the separation of proteins, 100 μ g in 20 μ l protein solution per tube was loaded on the gel (100 x 5 mm), and the entry of the sample into the gel was initiated at 0.5 mA per gel. After the dye had entered, the current was raised to 2.0 mA per tube, and the gel run at 32 mA at 60 V for 16 tubes for 4–5 hours at 10 °C. The gels were stained with Coomassie Brillant Blue R – 250 and protein samples were identified densitometrically by electrophoretic mobility of protein bands (Weber & Osborn, 1969). The identified MyHC protein bands were sliced and dissolved in 25% pyridine solution. The eluted dye was analysed for absorbance at 605 nm. The amount of protein was determined by quantitation of the extracted Coomassie Brillant Blue. Dye binding capacity of individual purified proteins was determined as the total absorbance/mg of protein (Fenner *et al*, 1975; Murakami & Uchida, 1985).

For liberation, the total amount of protein from gel, hydrogen peroxide was added, and the specific protein fraction was analysed for radioactivity and protein.

7. Recovery and Hydrolysis of MyHC Protein for Amino Acid Analysis

The MyHC protein was electroeluted from 10% SDS-PAGE according to Hunkapiller *et al*, 1983. After staining with Commassie Brillant Blue R-250 and detection on 10% SDS-PAGE the protein band was sliced and minced with a 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 NH₄HCO₃) for 1.0 h, the electroelution was continued in the dialysing bag, by using horizontal electrophoresis (Gel Electrophoresis Apparatus GNA – 100, Pharmacia, 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 μ l) and the gel pieces were removed by centrifugation (14,000 g). Residual SDS was removed by the following dialysis in 1.0 ml dialysis buffer (0.02% SDS in 0.01 M NH₄HCO₃) and rinsing the samples twice with 1.0 ml deionized water by centrifugation in microcentrifuge tube (10,000 D) and MyHC protein was washed from filter with 800 μ l deionized water.

Eluent fractions containing MyHC protein (200 μ l) were evaporated with a nitrogen stream, and the protein-bound amino acids from 10 μ g samples were liberated by hydrolysis at 110 °C for 18 h in 200 μ l 6 N HCl in the nitrogen area, and HCl was evaporated with nitrogen.

The leucine quantity in MyHC hydrolysate was determined by using an ultra rapid and sensitive high-pressure liquid chromatography (HPLC) method for measuring individual free amino acids in biological fluids by Graser *et al* (1985) by employing pre-column derivation with *o*-phthaldialdehyde/3-mercaptopropionic acid and using $3-\mu$ m-particle-size reversed-phase columns (Hyperchrome, Spherisorb ODS II, 3μ m, 125×4.6 mm with guard columns 10 $\times 4.6$ mm, 5μ m; 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 protein fraction was analysed for radioactivity and protein, as well as amino acid leucine.

8. Separation of MyHC Isoforms

Muscle pieces were pulverized under liquid nitrogen, and crude extracts of myofibrillar proteins were prepared by homogenizing the muscle powder 1:7 (wt/vol) in the buffer containing: 0.3 M KCl, 0.1 M KH₂PO₄, 50 mM K₂HPO₄, 10 mM ethylendiaminetetraacetic acid (EDTA), pH 6.5. After extracting for 15 min at 0 °C, the homogenate was centrifuged at 11,000 g for 10 min. The supernatant fraction was diluted at 1:1 (vol/vol) with glycerol and stored at -20 °C. (Bär & Pette, 1988). Protein concentration was measured by the method of Lowry *et al* (1951). Aliquots containing 0.5 μ g of protein in 10 μ l were loaded on the gel after being incubated for 10 min at 65 °C in lysis buffer containing: 10% (vol/vol) glycerol, 5% (vol/vol) 2-mercaptoethanol, 2.3% SDS, 0.05% bromophenol blue, 60 mM TRIS-HCl, pH 6.8 (Bär & Pette, 1988). MvHC isoforms were separated by 5–8% SDS-PAGE (Bär & Pette, 1988) using 1.0 mm thick gel (Sugiura & Murakami, 1990). Electrophoresis lasted for 24 h at 120 V. Gels were silver-stained by the method of Oakley (Oakley et al, 1980). Immunoblotting analysis was used to identify of different MyHC isoforms. Protein isoform bands were analysed densitometrically by Image Master[®] 1D program, version 3.0 (Amersham Pharmacia Biotech) and the percentage distribution of the MyHC isoforms was evaluated.

9. Immunoblotting

MyHC isoforms were identified by immunodetection by using the Western blotting protocol. The 8% SDS-PAGE gels, by the method of Talmadge and Roy (1993), with 0.15 μ g myofibrillar protein per lane, were used to separate MvHC isoforms for immunodetection. Gels were electrophoresed with the minigel apparatus (Bio-Rad) in running buffer (50 mM TRIS, 75 mM glycine) at 70 V for 12 h. After electrophoresis MyHC proteins' bands were transferred to polyvinylidene difluoride transfer (PVDF) membrane (Polyscreen[™] PVDF) membrane, Biotechnology Systems, NEN Research Products Du Pont) in transfer buffer (20% methanol in running buffer with 0.037% SDS) for 3 h at 44 V, 380 mA, using platinum-coated plate electrodes (Trans Blot Cell, Bio-Rad) at 5 °C. Blots were blocked for 1 h at room temperature on an orbital shaker with 3% bovine serum albumin (BSA) in phosphate buffered saline – TWEEN-20 (PBS-T-20) consisting of 20 mM NaH₂PO₄·H₂O, 80 mM Na₂HPO₄, 100 mM NaCl, 0.1% TWEEN-20. After rinsing and washing $(2 \times 5 \text{ min and } 1 \times 15 \text{ min})$ with PBS-T-20 the blots were incubated for 45 min at the 22 °C with diluted monoclonal antibody (6 μ l per 3 ml 10% BSA in PBS-T-20) recognizing skeletal slow- type MyHC isoforms (Clone WB MHCs) and fast-type MyHC isoforms (Clone WB MHCf), purchased from Novocastra Laboratories (Newcastle upon Tyne, UK).

After washing with PBS-T-20 (2 x 25 min) the blots were incubated for 45 min at 22°C with biotinylated secondary antibody (anti-mouse immunoglobulin-G) 0.5 μ l per 7 ml 1% BSA/PBS-T20. The bands were visualized by using the enhanced chemiluminesence (ECL) technique, according to the ECL™ Western blotting protocols (Amersham, Life Science). Before the detection of protein bands, the membrane was washed $(1 \times 15 \text{ min and } 4 \times 5 \text{ min})$ in fresh changes of washing buffer (PBS-T-20). For the detection of protein bands, membranes were exposed for 1 min to ECL Western blotting detection reagents solution and light emission was detected after a short exposure (15 min) to sensitive autoradiographic film (Reflection[™] NEF - 496, NEN Research Products Du Pont). The most effective exposure time was set out by trial. The same membrane was used twice, first to recognize slow-type MyHC isoform and then after the complete removal of primary and secondary antibodies from the membrane by boiling for 2 h in stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM TRIS-HCl pH 6.7), second to recognize skeletal fast-type MyHC isoforms. The complete removal procedure removes the antibodies and detection reagents without damaging the proteins. After the removal of previous antibodies, blots were blocked and immunodetection was carried out as described above. The relative protein content was evaluated densitometrically.

10. Turnover Rate of MyHC

The relative specific activity (RSA) which characterises the turnover rate of MyHC protein fraction was calculated as a ratio of the specific activity of the protein fraction (Sb) to the specific activity of mixed muscle cell protein (Sa) (Schreurs *et al*, 1981; 1985) and was expressed in percentages.

11. Fractional Synthesis Rate of Muscle Proteins

Synthesis of protein (mixed muscle cell protein, myofibrillar protein, MyHC) was expressed as fractional synthesis rate (Sugden & Fuller, 1991). The fractional rate of protein synthesis (Ks, expressed as a percentage of the protein synthesized per day) in each fraction was then calculated from the following formula: $Ks = 100 \times Sb/Sa \times t$, where Sa and Sb are the specific radioactivities of mixed muscle cell protein and protein-bound leucine and t is the incorporation time in days.

12. Statistical Analysis

Standard statistical methods were used to calculate means, standard errors (SE), and Pearson product moment correlation coefficients. The data were then analysed using analysis of variance (ANOVA). The p<0.05 criterion was used to establish statistical significance.

RESULTS

1. Effect of Character of Resistance Training on the Skeletal Muscle

The main characteristic of resistance exercise training is the ratio between the used volume and power. As seen in Figure 1A, the training protocols are in this aspect significantly different. The number of repetitions (Figure 1B) provides some information about the training volume, too. Relatively high volumes of resistance training lead to a decrease in absolute grip strength (Figure 2B). As seen in Figure 2B, in Gr 3 where the same training power and volume was applied instead of 6 weeks for 4 weeks, grip strength decreased significantly. At the same time there was no significant difference between resistance trained groups when carrying maximal weight to the height of 1.5 m during 5 sec (Figure 2A).

2. Effect of Mechanical Loading on the Hypertrophy of Skeletal Muscle

30 days after tenotomy of the *m. gastrocnemius*, the body mass of the rats had not changed. The mass of *m. plantaris* had increased in young rats (by 40% \pm 8%) and in old rats (24.8% \pm 5.4%). One month after RT the mass of *plantaris* muscle in young and old rats had increased 10% \pm 4% and 18% \pm 5%, respectively. The effect of CH simultaneously with CH and RT increased the mass of *m. plantaris* more than CH did. The ratio of total work to power was maintained at the same level in old and young rats during the training period. The total work during RT increased by 17.6% \pm 3% in old animals in comparison with young RT group because of the higher b.w. of the old animals.

In adult rats resistance training caused the hypertrophy of the *plantaris* muscle in Gr1 by 6.1%, in Gr 2 12% and in Gr 3 the muscle weight decreased 2.5% in comparison with the control group. There is no correlation between the state of hypertrophy of the *plantaris* muscle and grip strength between RT groups.



Figure 1. Characteristics of resistance training protocols

A - Dynamics of ratio in training volume and power (J/W) during the training period
B- Dynamics of repetitions during the training period
Gr 1 - Group 1. Three training days per week, six-week training period

Gr 2 - Group 2. Six training days per week, six-week training period

- Gr 3 Group 3. Six training days per week, four-week training period
- *** p<0.001 in comparison with Group 1

in comparison with Gr 2



Figure 2. Maximal carried weight and hindlimb grip strength in resistance trained rats

A – Maximal carried weight to the height of 1.5 m during 5 sec B – Hindlimb grip strength

Contr – Control group

Gr 1 – Group 1. Thee training days per week, six-week training period

Gr 2 – Group 2. Six training days per week, six-week training period

Gr 3 – Group 3. Six training days per week, four-week training period p = -p < 0.05

**
$$-p<0.01$$
 in comparison with control group

- p < 0.001 in comparison with Gr 3

3. Role of Satellite Cells in the Hypertrophy of Muscle Fibres

To clarify the role of satellite cells in the process of muscle hypertrophy during mechanical loading, the incorporation of ³H-thymidine into DNA was used. As Figure 3C shows, the incorporation of ³H-thymidine was observed in some nuclei of mature muscle fibres. As ³H-thymidine is not incorporated into the nucleus of mature muscle fibres, it is only possible after fusion of satellite cells with damaged muscle fibres. Between mature fibres there are some thin newly formed fibres (Figures 3A, B, C). This process shows that satellite cells participate in the process of mechanically induced muscle hypertrophy. Recruitment of glycolytic muscle fibres during resistance training is different in different muscles and their parts.

On some muscle fibres terminals of neuromuscular junctions are filled with acetylcholine vesicles, in others there are only a few (Figure 4). A decrease in acetylcholine vesicles in terminals of neuromuscular junction, characterizes exhaustion of the synaptic part, but probably also motoneurons. Ultrastructural changes in neuromuscular junctions after resistance training also characterize the selective hypertrophy of muscle fibres in working muscles.



Figure 3. Effect of resistance training on the glycolytic muscle fibres

A and B – A newly formed thin muscle fibre (1) between thick, mature fibres (2). 3 - capillary; 4 - silver granules (phon level); 5 - nucleus of muscle fibre; 6 - myofibrils.

C – Silver granules on the muscle fibre nucleus and nucleolus (7) in mature fibre 48 hours after infusion of 3 H thymidine.

Magnification A - 5 200 x; B - 10 000 x; C - 26 400 x.



Figure 4. Neuromuscular junction of glycolytic muscle fibre after resistance training

1 – axon terminal; 2 – few synaptic vesicles; 3 – Schwan cell; 4 – coated vesicles; 5 – dilated tubule of sarcoplasmic reticulum. Magnification 25 000 x.

4. Fractional Synthesis Rate of Mixed Muscle Protein and MyHC

The mixed muscle protein synthesis rate per day in *m. plantaris* shows a significant increase after CH and its combination with RT (CH + RT) in comparison with the control group and the resistance trained group (Figure 5). The daily fractional synthesis rate of MyHC also shows a significant increase after all types of mechanical loading (Figure 6).



Figure 5. Fractional synthesis rate of mixed muscle protein in the *plantaris* muscle after mechanical loading

Control – control group

RT - resistance training group

CH – compensatory hypertrophy group (surgical tenotomy of the synergist muscle)

CH + RT – combination of compensatory hypertrophy and resistance training group (combination of synergistic tenotomy and resistance exercise)

* - p < 0.05 in comparison with the control group

#-p < 0.05 in comparison with resistance training



Figure 6. Fractional synthesis rate of MyHC in the *plantaris* muscle after mechanical loading

Control – control group

RT - resistance training group

CH – compensatory hypertrophy group (surgical tenotomy of the synergist muscle)

CH + RT – combination of compensatory hypertrophy and resistance training group (combination of synergistic tenotomy and resistance exercise)

* - p < 0.05 in comparison with the control group

5. Fractional Synthesis Rate of Myofibrillar Proteins in Young and Old Rats

The fractional synthesis rate of myofibrillar proteins in the *plantaris* muscle was significantly more intensive in young rats than in old rats (Figure 7). Simultaneous CH and RT influenced the fractional synthesis rate in plantaris in both age groups, RT only in old rats in comparison with subsequent controls (Figure 7). The fractional synthesis rate of total muscle protein in the *plantaris* was $5.1\% \pm 0.1\%$ per day in young rats and, $3.8\% \pm 0.6\%$ per day, in old rats.



Figure 7. Effect of different modes of mechanical loading on the fractional synthesis rate of myofibrillar proteins in the *plantaris* muscle in young and old rats

RT – resistance exercise training group CH – compensatory hypertrophy group CH + RT – combination of compensatory hypertrophy plus resistance exercise training group young – 12-week-old animals at the beginning of experiments old – 80-week-old animals at the beginning of experiments * – p<0.05 in comparison with the control group π – p<0.05 in comparison with subsequent young rats π – p<0.01

6. Effect of Mechanical Loading on the Synthesis Rate of MyHC Isoforms

Our results show that incorporation of leucine is different between individual MyHC isoforms and depends on the applied stimulus. Incorporation of the radioactive label into MyHC I and IIa is more intensive than into MyHC IIb and IId (Figure 8).

Compared with the control group the incorporation of leucine into MyHC IId isoform increased after resistance exercise training (Figure 8). The incorporation of radioactive label into MyHC I and IId isoform increased in comparison with the control group during compensatory hypertrophy of *m. plantaris* (Figure 8). At the same time, the incorporation of leucine into MyHC IIb decreased. The combination of CH and RT increased leucine incorporation into MyHC I and IId and decreased its incorporation into MyHC IIb isoform (Figure 8).

7. Effect of Mechanical Loading on the Composition of MyHC Isoforms

The relative content of MyHC I isoform increased during all types of mechanical loading (Figure 9 and 10). At the same time the relative content of MyHC IIa isoform increased only during CH (Figure 10). In the case of CH + RT the relative content of MyHC IIa isoform decreased (Figure 10). The increase of the relative content of MyHC IId isoform correlated (r=0.81) with the muscular hypertrophy level. The relative content of MyHC IIb isoform decreased in all types of mechanical loading. However, in comparison with CH, the combination of CH and RT increased the relative content of MyHC IIb isoform in the *plantaris* muscle (Figure 10).



Figure 8. Incorporation of L [4.5³ H] leucine into MyHC isoforms in the *plantaris* muscle after mechanical loading

Control – control group

RT – resistance training group

CH – compensatory hypertrophy group (surgical tenotomy of the synergist muscle)

 $\rm CH + RT$ – combination of compensatory hypertrophy and resistance training group (combination of synergistic tenotomy and resistance exercise)



Figure 9. Changes in composition pattern of MHC isoforms in the *plantaris* muscle during mechanical loading in young and old rats

1 – young contol, 2 – old control, 3 – old CH, 4 – old RT, 5 – old CH + RT, 6 – marker proteins

RT - resistance exercise training group

CH – compensatory hypertrophy group

CH + RT – combination of compensatory hypertrophy plus resistance exercise training group



Figure 10. Relative content of MyHC isoforms in the *plantaris* muscle after mechanical loading

Control – control group

RT - resistance training group

CH – compensatory hypertrophy group (surgical tenotomy of the synergist muscle)

CH + RT – combination of compensatory hypertrophy and resistance training group (combination of synergistic tenotomy and resistance exercise) * -p < 0.05

in comparison with the control group

$$** - p < 0.01$$

in comparison with the resistance training group

- p<0.01 J

m - p < 0.05 in comparison with the compensatory hypertrophy group

8. Effect of Mechanical Loading on the Composition of MyHC Isoforms in Young and Old Rats

There was a significant difference in the composition of MvHC isoforms between young and old rats (Figures 9 and 11), mainly related to the proportion of MyHC I (Figure 11A). The relative content of the MyHC I isoform in the *plantaris* muscle depends on the body mass (r=0.85) only in old controls. The relative content of the MyHC I isoform increased during all modes of mechanical loading in young and old animals. However, in comparison with the subsequent control groups there was a more significant increase in the MyHC I isoform in young animals (Figure 11A). As Figure 11B shows, the relative content of the MyHC IIb isoforms was significantly higher in young sedentary rats than in old ones. CH decreased the content of MyHC IIb isoform in both young and old groups in comparison with the subsequent control groups. In young CH animals the relative content of the MyHC IIb isoform decreased approximately three times. In old animals the decrease was much smaller (Figure 11B). In young RT and CH plus RT animals, the relative content of the MyHC IIb isoform decreased significantly (Figure 11B). In old RT and CH plus RT animals, the relative content of the MyHC IIb isoform increased significantly (Figure 11B).

Figure 11C shows that the relative content of the MyHC IId isoform in the control group increased with age. In young animals the relative content of the MyHC IId isoform significantly increased with CH, RT, and CH plus RT. In old animals the relative content of the MyHC IId isoform decreased with RT and, CH plus RT animals. Despite these differences between young and old animals, the relative content of the MyHC IId isoform significantly increased with CH in both age groups, and CH had a considerably stronger effect on the MyHC IId content in old animals (Figure 11C). No significant difference in the relative content of the MyHC IIa isoform was noted between young and old rats (Figure 11D). During the RT and CH plus RT, the relative content of MyHC IIa isoforms in young rat *plantaris* decreased. In old rats no changes were noted with RT or stimultaneous CH and RT in the relative content of the MyHC IIa isoform (Figure 11D). The relative content of MyHC IIa with CH increased significantly in young animals, and decreased in old animals (Figure 11D).



Figure 11. Relative content of MHC isoforms in the *plantaris* muscle during mechanical loading in young and old rats. A – MHC I; B – MHC IIb; C – MHC IId; D – MHC IIa

 $\begin{array}{l} * & -p < 0.05 \\ *^{*} - p < 0.01 \\ \end{array} \right\} \text{ in comparison with the subsequent control group} \\ *^{*} - p < 0.05 \\ \text{in comparison with the subsequent young rats} \\ \hline \\ \text{RT} - p < 0.01 \\ \text{RT} - resistance exercise training group; CH - compensatory hypertrophy group;} \end{array}$

CH + RT - combination of compensatory hypertrophy plus the resistance exercise training group

9. Effect of Character of Resistance Training on the Relative Content of MyHC Isoforms in Adult Rats

This experiment shows that resistance training with lower repetitions, lower training volume and the same power (Group 1) is leading to the increase of MyHC IIb isoform and decrease of MyHC I and IIa isoform in *plantaris* muscle (Figure 12). As shown in Figure 12 MyHC IIb isoform is sesnitive to the high training volume, but not to the power. MyHC IId isoform is increasing independently of training volume and power (Figure 12).



Figure 12. Changes in the realtive content of MyHC isoforms during resistance training with different ratios of volume and power

 $\begin{array}{l} \mbox{Gr 1} - \mbox{Group 1. Three training days per week, six-week training period} \\ \mbox{Gr 2} - \mbox{Group 2. Six training days per week, six-week training period} \\ \mbox{Gr 3} - \mbox{Group 3. Six training days per week, four-week training period} \\ \mbox{**} & -p < 0.01 \\ \mbox{***} & -p < 0.01 \\ \mbox{##} & -p < 0.01 \\ \mbox{##} & -p < 0.01 \\ \mbox{in comparison with Gr 1} \\ \mbox{###} & -p < 0.001 \end{array} \right)$

10. Effect of Mechanical Loading on the Turnover Rate of MyHC

In young rats MyHC in the *plantaris* muscle turned over faster than in old rats (Figure 13). Despite this significant difference between the two age groups, MyHC turns over faster in young and old groups during RT and CH combined with RT (Figure 13). With CH, MyHC turns over significantly faster only in young animals. The biggest difference between the MyHC turnover in young and old rats was found during CH, where the MyHC of young rats turned over approximately twice as fast as in old rats.



Figure 13. Turnover rate of MHC in *plantaris* muscle during mechanical loading in young and old rats

RT - resistance exercise training group

CH - compensatory hypertrophy group

 $CH + RT - combination of compensatory hypertrophy plus the resistance exercise training group % <math display="inline">\left({{\left({RT - L} \right)} \right)^2} \right)$

young -12 week-old animals at the beginning of experiments

$$\begin{array}{c} \text{old} - 80 \text{-week-old animals at the beginning of experiments} \\ * & -p < 0.05 \\ *^{*} & -p < 0.01 \end{array} \right\} \text{ in comparison with the subsequent control group} \\ \begin{array}{c} \text{animals at the beginning of experiments} \\ \text{in comparison with the subsequent control group} \\ \text{animals at the beginning of experiments} \\ \text{at the beginning of experming of experments} \\ \text{at the begin$$

DISCUSSION

Resistance training (RT) induced changes at the muscle-fibre level have been related to hypertrophy in different types of muscle fibres (Mastropaolo, 1992). Mostly it has been shown that RT causes an increase in the diameter of glycolytic and oxydative-glycolytic types of fibres. In some studies, however, type IIB fibers (glycolytic) have been shown to decrease (Andersen & Aagaard, 2000).

The development mechanism of muscle fibre hypertrophy during mechanical loading in adult animals and humans is still open. The repetition regime in RT seems to play a important role in the development of muscle fibre hypertrophy. It has been shown that high repetitions in RT did not cause any significant hypertrophy (Campos *et al*, 2002).

The increased number of satellite cells in powerlifters shows that satellite cells will make muscle more responsive to training (Thornell *et al*, 2003). An increase in satellite cells is related to several factors expressing different genes, and fast-twitch muscle hypertrophy (Sutrave *et al*, 1990; Hespel *et al*, 2001; Carson *et al*, 2002). It has been shown that IGF-1 is involved in the hypertrophy of muscle fibres via stimulation of differentiation in satellite cells. The MGF level increases with the number of satellite cells in mature muscle fibres (Haddad & Adams, 2001).

The results of our study show that in adult rats RT causes skeletal muscular hypertrophy in two ways. First, damaged mature fibres regenerate as a result of fusion with satellite cells. It is proved by incorporation of ³H thymidine into the nucleus of the muscle fibre. As ³H thymidine is not incorporated into the nucleus of a mature muscle fibre, the only way of incorporation is via satellite cells. The second way is activation of satellite cells under the basal laminae of muscle fibres during RT. Satellite cells divide and later myosymplasts fuse with each other and form myotubes. As noted, myotubes are a source of forming new muscle fibres during RT. Thus, even in adult rats hyperplasia palys a certain role in the process of muscle hypertrophy during RT.

It is well known that different modes of mechanical loading resulted in the selective up- and downregulation of MyHC isoforms in fast-twitch-skeletal muscle in humans and animals. One of the aims of this work was to develop an animal model that mimics human resistance exercise. Comparison of different resistance training protocols (the main difference was in the ratio of exercise power to volume) enabled me to find a RT programme, that increased the relative content of most of all in fast-twitch muscles MyHC IIb isoforms.

The increase in the MyHC IIb isoform had a positive correlation with hindlimb grip strength and a negative correlation with the training volume and the number of repetitions per training session. A low number of repetitions during the training session and a low volume of RT (three training days per week) causes relatively small hypertrophy of muscle. However, the highest increase of grip strength and increase in the relative content of the MyHC IIb isoform in fast-twitch muscles. It seems that both in the case of resistance and endurance training the increase in the training volume decreases the relative content of the MyHC IIb isoform in fast-twitch skeletal muscles.

The examination of the mechanisms associated with activity-induced shifts in myosin expression is the key to understanding plasticity of skeletal muscle as the hypertrophied muscle has adapted to a chronic overload via an alteration in its phenotype (Pette, 2001). The mechanical loading increases muscle mass. The mechanisms involved in regulating changes in myosin expression and muscle mass may have differential sensitivities to mechanical loading (Hernandez *et al*, 2000).

The synthesis rate of MyHC in skeletal muscle is on average 28% slower than that of mixed muscle protein, and contributes only 18% to the synthesis rate of mixed muscle protein although MyHC comprises 25% of the muscle protein content. Comparison of the fractional synthesis rate of mixed muscle protein and MyHC shows that during mechanical loading a higher magnitude of changes in the synthesis rate of MyHC could be offset by changes in muscle proteins other than MvHC, which turn over faster than MvHC and do not reflect the synthesis rate of contractile proteins. It is known that there are at least four adult MyHC isoforms expressed in rat fast-twitch plantaris muscle (Fauteck & Kandarian, 1995; Bottinelli, 2001). As the synthesis of mixed muscle protein does not reflect changes in individual proteins, the synthesis rate of MyHC in skeletal mucle likewise does not provide any useful information per the understanding of up- and down-regulation mechanisms of the MyHC isoforms under mechanical loading. This study showed for the first time that the synthesis rate of MyHC I and IIa isoforms is relatively similar. In comparison with IIb and IId, the synthesis rate of the MyHC I isoform is significantly faster. Although CH and its combination with RT increased the synthesis of MyHC I isoforms, it does not seem that isoforms with a faster synthesis rate are more sensitive to loading as was found also in the IId isoform. The findings of this study showed a decrease in the relative content of the MvHC IIB isoform during all mechanical loadings, except for RT with a low training volume.

Hypertrophied *plantaris* muscles demonstrated a fast-to-slow shift in MyHC composition as evidenced by increased I, IIa and IIx MyHC isoforms and by decreased IIb MyHC isoform expression (Demirel *et al*, 1999). The results of this study show that mechanical loading up-regulates first of all the relative protein content of the MyHC I and MyHC IId isoforms in muscle. It seems likely that the volume of mechanical loading per day is the main stimulus for the upregulation of the relative content of MyHC I and IId isoforms in *m. plantaris*. At the same time the MyHC IIb isoform is down-regulated. Even vigorous short (5 s) bouts of resistance exercise and their small total number per day did not lead to the up-regulation of MyHC IIb isoforms in the *plantaris* muscle. Only very few repetitions with a relatively high power, and three training days per week up-regulated the MyHC IIb isoform in fast-twitch

muscles. In the case of a combination of CH and RT the relative content of the MyHC IIb isoform increased significantly in comparison with CH.

The claim by Caiozzo et al, (1996; 1997) about the role of mechanical loading and stimulation frequency is correct in the case of total MyHC upregulation, but at the same time it does not characterize the dynamics of all the isoforms including the MyHC IIb isoform. Mechanical loading of a different character leads to different fast-to-slow shifts in the composition of the fasttwitch muscle. Andersen and co-workers demonstrated on humans that heavy resistance training decreases the amount of MvHC IIx and increases the content of MyHC IIa (Andersen & Aagaard, 2000). There is also excellent agreement between MyHC concentrations measured on the whole muscle level (Tikunov et al. 2000). Although it is generally accepted that mechanical loading changes the MyHC isoform pattern, our findings demonstrate that RT and CH of the muscle after tenotomy of the synergist have different effects on the modulation of the composition of MyHC IIa and IIb isoforms in skeletal muscle. These differences in MvHC isoform composition are related to differences in the synthesis rate of MyHC IIa and IIb isoforms in the plantaris muscle. The synthesis rate of the MvHC IId isoform seems to be the most sensitive to all types of mechanical loadings. Although the relative content of the MyHC IIb isoform decreases during most types of mechanical loading, the combination of compensatory hypertrophy and RT increases the isoform content in comparison with CH. This knowledge may be helpful when combining long-lasting mechanical loading with resistance exercise in order to avoid transformation of muscle contractile properties from fast to slow. This may be particularly useful in rehabilitation when combining the development of compensatory hypertrophy with resistance exercise. This fact, shown in this work for the first time, may be important both in exercise training and rehabilitation. In most cases the synthesis rate and relative content of the two fastest isoforms of MyHC IIb and IId are regulated in different directions during mechanical loading. The combination of CH and RT in comparison with CH increased the relative content of MyHc IIb isoform in the *plantaris* muscle.

Age-Related Differences in Skeletal Muscle and Adaptation to Mechanical Loading

Decrease in muscle strength has been found to be associated with older age. Beginning in midlife, ageing is associated with a time-dependent loss of muscle mass. This is a major cause of disability, frailty, and loss of independence in the elderly. Loss of muscle mass is mainly caused by a loss of fast-twitch muscle fibres and a decrease in the fastest II type MyHC isoforms. The decrease in the muscle mass is related to the decrease in synthesis and an increase in degradation rate of muscle proteins. MvHC isoform composition during RT in humans and animal has shown a decrease in IIb isoforms in animals and IId isoforms in humans. It has been shown that MvHC IIa isoforms increase, but the composition of the MyHC I isoform does not change during RT in humans. In our experiment RT caused a decrease in the MyHC IIb isoform in the fasttwitch skeletal muscle of young rats. However, contrary to earlier results (Caiozzo et al, 1997) the MyHC I isoform increased in young and old rats similarly in all types of mechanical loading. The MHC IId isoform in young rats increased with RT (Caiozzo et al, 1997). The more intensive expression of MvHC I isoform resulted in increased isometric activity (Diffee et al, 1993), which is an integral component of extra weight bearing and postural control, associated with RT on the vertical treadmill. The changes in the expression of MyHC IIb and MyHC IId isoforms in young animals may have resulted from altered pre-translational mechanisms as the RT programme produced a rapid elevation in the fast-type MyHC IId mRNA isoform and the corresponding repression of the IIb MyHC mRNA isoform (Caiozzo et al, 1996).

It was found that the proportions of MyHC I and MyHC IId isoforms are significantly higher in old sedentary rats than in young ones, and the proportion of the MyHC IIb isoform proportion is significantly higher in *m. plantaris* in young rats than in old ones. In comparison with the control groups, all types of mechanical loading – CH, RT, and CH plus RT – significantly increased the proportion of MyHC I and MyHC IId isoforms in young rats. RT decreased the proportions of MHC IIB and MHC IIA in young and the proportions of MyHC IId in old rats. CH decreased the proportion of MHC IIb in young and old, and MyHC IIa in the muscle of old animals.

On the basis of CH in *m. plantaris* it seems that skeletal muscles of young rats are more sensitive to continuous mechanical loading induced by tenotomy of *m. gastrocnemius*. This might be due to a more intensive rate of muscleprotein synthesis in young animals (Hasten et al, 2000). These findings are consistent with an early study in humans in which the rate of muscle protein synthesis in young and elderly skeletal muscle was highly sensitive to the resistance exercise-induced stimulus (Trappe et al, 2002). Although the abovementioned authors examined synthesis in total muscle protein, this study indicates that the same principle is applicable to the synthesis of myofibrillar proteins. RT increased the mass of *m. plantaris*, as well as the fractional synthesis rate of myofibrillar proteins in old rats and in conjunction with CH. The MyHC of the skeletal muscle of young and old rats turned over much faster after RT, and this is probably due to the increase in the proportion of MyHC I and IId isoforms and the decrease in the MyHC IIb isoforms. It has been shown in previous papers that MyHC turned over faster in type I and IIA muscle fibres than in IIB fibres (Seene & Alev, 1991), and the turnover rate of skeletal muscle MyHC depends on the functional activity of the muscle (Seene et al, 1986). The turnover rate of MyHC in skeletal muscle seems to be related to changes in the composition of the MyHC isoform. The faster MyHC turnover rate in the skeletal muscle of young rats is linked to the more intensive fractional synthesis rate of myofibrillar proteins. RT is a strong stimulus for the MyHC metabolism of skeletal muscle in young and old rats. RT increased significantly the fractional synthesis rate of myofibrillar proteins in the fast-twitch skeletal muscle in old rats. MyHC in *m. plantaris* of young rats turned over faster in all the types of mechanical loading, in old rats during RT and CH plus RT. The slowdown of the turnover rate by approximately 30% with age is caused both by the age-related slowdown of protein synthesis and the intensification of protein degradation. The same principle applies to the changes in the MvHC turnover rate in the case of mechanical influences. Although mechanical influences change the turnover rate in both age groups, the turnover rate changes in old age are relatively smaller than in young animals. One reason for this might be the age-related decrease in the number of ribosomes and increase in the number of lysosomes in muscle cell. It is incorrect to assume that the MyHC turnover rate depends on the relative concentration of type I and type IIa MyHC isoforms in the muscle since the relative content does not reflect the absolute content of the corresponding isoforms. Moreover, the MyHC isoform content might not be directly connected with the fibre composition of muscle but also depends on the polymorphism of muscle cell. This has been shown by studies which found that there was a substantial increase in the MyHC IId isoforms in skeletal muscle. Single fibre analyses demonstrated that many fasttype 2B fibres contained small amounts of fast-type IId MyHC isoforms. Although RT altered the bias of the distribution of fast-type IIb and IId MvHC isoforms in IIB fibres. RT did not increase the number of fibres that could be categorized as exclusively fast-type 2D fibres.

In conclusion, fractional synthesis rate of myofibrillar proteins, relative content of MyHC isoforms, and MyHC turnover rate are all age-specific and mechanical-load- specific. The relative content of MyHC I and IId isoforms increased with age and MyHC IIb decreased. RT decreased the relative content of the MHC IId isoform the muscle of old rats. Compensatory hypertrophy decreased the relative content of MHC IIb and MyHC IIa isoforms in the muscle of old animals. Simultaneous CH and RT increased the proportion of the MyHC IIb isoform and decreased MyHC IId in the muscle of old animals. RT also prevents the age-related decrease in the relative content of the MyHC IIb isoform in the *plantaris* muscle.

CONCLUSIONS

- 1. The response of fast-twitch skeletal muscle to increased mechanical activity depends on the ratio of the power to volume of the mechanical load and the age of the experimental animals. The skeletal muscle of young animals responded with highest hypertrophy to the continous low-power load; in old animals it responded to the resistance training. In the process of resistance training the activation of satellite cells caused muscle hypertrophy; their fusion with damaged mature fibres or formation new fibres palys an important role.
- 2. All modes of mechanical loading increased the relative content of the slowest myosin heavy chain I isoform in fast-twitch skeletal muscle more in young than in old animals. The relative content of the fastest myosin heavy chain IIb isoform in young animals decreased during all modes of higher volume mechanical loading. However, in old animals it increased during resistance training.
- 3. In the fast-twitch skeletal muscle of young and adult animals, the myosin heavy chain IIb isoform is very sensitive to the volume of resistance training and its relative content increased only in the case of low-volume, high-power resistance training.
- 4. In all age groups resistance training increased the synthesis of contractile proteins synthesis and the turnover rate. Myosin heavy chain IIb isoforms, the synthesis rate of which was the lowest among others, did not increase during resistance training.
- 5. All modes of mechanical loading increased the relative content of dominating myosin heavy chain IId isoform in the *plantaris* muscle of young and adult animals, and the increase showed a correlation with the capacity to carry maximum weight. Increase in the relative content of the myosin heavy chain IIb isoform shows a positive correlation with grip strength.

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SUMMARY IN ESTONIAN

Mehaanilise koormuse ja vananemise mõju müosiini raskete ahelate uuenemisele kiiretes skeletilihastes

Käesoleva töö eesmärk oli uurida kontraktiilsete valkude sünteesi ja müosiini raskete ahelate transformatsiooni kiiretes skeletilihastes erineva iseloomuga mehaanilise koormuse ja vananemise puhul. Töös käsitletakse satelliitrakkude osa hüpertroofia tekkemehanismis jõutreeningu puhul, samuti erineva iseloomuga mehaanilise koormuse ja vananemise mõju kontraktiilsete valkude sünteesile ning uuenemisele kiiretes skeletilihastes. Töös otsitakse seoseid erinevate mehaaniliste koormuste poolt põhjustatud müosiini raskete ahelate isovormide suhtelise sisalduse muutuste ja roti tagajäsemete haardejõu ning maksimaalse raskuse tõstmise vahel.

Töö tulemused näitavad, et kiirete skeletilihaste vastusreaktsioon mehaanilisele koormusele sõltub suuresti tehtud töö mahu ja võimsuse suhtest. Noorte katseloomade lihas reageerib kõige suurema hüpertroofiaga madala võimsusega suuremahulisele koormusele, vanade katseloomade lihas aga suure võimsusega jõutreeningule.

Jõutreeningu poolt põhjustatud lihashüpertroofia tekkes etendab olulist osa satelliitrakkude aktiveerumine, nende liitumine kahjustunud täiskasvanud lihaskiududega või uute lihaskiudude moodustumine. Kõik kasutatud mehaanilised koormused põhjustasid noorte katseloomade kiiretes skeletilihastes vanade katseloomadega võrreldes müosiini raske ahela kõige aegalsema isovormi ehk I isovormi suhtelise sisalduse olulise suurenemise.

Samal ajal müosiini kõige kiirema isovormi ehk IIb isovormi suhteline sisaldus langes noortel loomadel kõige enam suuremahulise koormuse puhul, vanadel aga suurenes jõutreeningu puhul.

Noorte katseloomade kiiretes lihastes on müosiini raske ahela IIb isovorm väga tundlik treeningumahu suurenemise suhtes. IIb isovormi suhteline sisaldus suureneb ainult väikese mahu ja suure võimsusega jõutreeningu puhul. Kontraktiilsete valkude sünteesi ja uuenemise kiirus tõusevad jõutreeningu puhul.

Müosiini raske ahela IIb isovormi sünteesi intensiivsus on teiste isovormidega võrreldes madalaim ega kiirene ka jõutreeningu puhul. Kõik kasutatud mehaanilised koormused suurendasid domineeriva müosiini raske ahela IId isovormi suhtelist sisaldust noorte ja täiskasvanud loomade kiiretes skeletilihastes ning eelmainitud müosiini raske ahela isovormi osakaalu suurenemise ja maksimaalse raskuse kandmise vahel ilmnes positiivne korrelatiivne seos. Müosiini raske ahela IIb isovormi osakaalu suurenemise ja tagajäsemete haardejõu vahel ilmnes samuti positiivne korrrelatiivne seos.

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PUBLICATIONS

Pehme A., Alev K., Kaasik P., Julkunen A., Seene T. The effect of mechanical loading on the MyHC synthesis rate and composition in rat plantaris muscle. Int. J. of Sports Medicine (accepted 2003, in press).

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Teadustegevus

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