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Relationships between glucocorticoid-caused atrophy and overload: muscle cell morphological characteristics and protein expression

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CONTENTS

ABBREVIATIONS

AFM – atomic force microscopy

CSA – cross-sectional area

DEX – dexamethasone

DEX+CH – dexamethasone-treatment and compensatory hypertrophy

EGTA – ethylene glycol tetraacetic acid

FT – fast-twitch

GAST – *gastrocnemius* muscle

GC – glucocorticoid

H&E – hematoxylin and eosin

MyHC – myosin heavy chain isoform

NPC – nuclear pore complex

PLA – *plantaris* muscle

SDS-PAGE – sodium dodecyl sulfate polyacrylamide gel electrophoresis

SOL – *soleus* muscle

ST – slow-twitch

UPP – ubiquitin-proteasome pathway

INTRODUCTION

Skeletal muscle is the most abundant tissue in the human body accounting for ~40% of the total body mass. It is not only the major site of metabolic activity but it is also the largest protein reservoir, serving as a source of amino acid to be utilized for energy production during periods of food deprivation (Nader, 2005).

All skeletal muscles have adaptive potential, which means that they are capable of modifying their structure in response to environmental changes (e.g. muscle training and detraining) (Bruton, 2002). In many disease states or unfavorable environmental conditions, skeletal muscle mass could be markedly reduced, a condition that may have devastating health consequences. In contrast, some forms of physical activity such as resistance training, can produce large increases in skeletal muscle mass (Pehme *et al*., 2004; Nader, 2005).

In general, muscle hypertrophy is the result of an increase in the size of the existing muscle fibers. Such increase is reflected by the increase in cross-sectional area of the muscle fibers, which in turn is a consequence of the accumulation of contractile proteins due to a reduction in the muscle fiber cross-sectional area (Goldspink, 1999; Nader, 2005).

In hypertrophy, the rate of synthesis is much higher than the rate of degradation of muscle contractile proteins, leading to an increase in the size or volume of an organ due to enlargement of existing cells. When a muscle remains in disuse for a long period, the rate of degradation of contractile proteins becomes greater than the rate of replacement, resulting in muscle atrophy (Boonyarom & Inui, 2006). Like hypertrophy the muscle atrophy is increasingly recognized as a purposeful biological compensatory mechanism (Hoffman & Nader, 2004).

Our muscle has been fine-tuned through hundreds of millennia to make the best decisions to adapt to environmental insults or challenges, such as starvation versus the need for increased strength. Though such environmental challenges are now less of a concern in many populations, we face the scientific challenge of learning enough about these responses to be able to counteract them in clinically important settings such as diabetes, immobility and space flight (Hoffman & Nader, 2004).

I REVIEW OF LITERATURE

1. Skeletal muscle atrophy

Skeletal muscle is the most abundant tissue in the human body and its normal physiology plays a fundamental role in health and disease. During many disease states, a dramatic loss of skeletal muscle mass (atrophy) is observed. In contrast, physical exercise is capable of producing significant increases in muscle mass (hypertrophy). Maintenance of skeletal muscle mass is often viewed as the net result of the balance between two separate processes, namely protein synthesis and protein degradation (Chacon Heszele & Price, 2004; Jackman & Kandarian, 2004; Nader, 2005).

Skeletal muscle is a highly plastic tissue, poised at all times to undergo significant gain or loss of mass, responding to fluctuating demand of mechanical work and variety of signals that relay the physiological state of the whole organism (Allen *et al*., 1999; Szewczyk & Jacobson, 2005). Adaptation reflects the plastic nature of muscle when placed under certain conditions, ranging from disuse to high-resistance exercise. Adaptation in either direction, seek to maintain balance between form and function. When muscles are prevented from doing the work for which they are designed, they wither and weaken. Muscle atrophy and fiber-type changes are related to changes in functional performance, as evidenced by measurements of endurance, velocity, strength, contractile force and mobility. Changes in workload, diseases and aging processes may actually diminish the number of muscle cells that can be recruited for functional activity. The key strategy from muscle atrophy is prevention – if muscle is kept active and not allowed to waste, the effects of injury and loss over time will be less deleterious. Atrophy contributes to weakness by diminishing the muscle fiber size, which reduces the generation of contractile force. Changes in fiber type that occur with atrophy also affect the ability of a muscle to sustain contraction. This phenomenon of weakness affects the muscle's ability to perform its normal work, which characterizes functional limitation. But, more important, this association is bidirectional. Functional limitation can result in the impairment of muscle atrophy because of the profound adaptability of muscles. When function changes, so does the structure of muscle. Determining which is antecedent and which is consequent is one of the challenges in rehabilitation if the circular nature of this relationship is to be disrupted and structure and function restored. These adaptations, in turn, alter the size and/or metabolic properties of the

muscle fibers, resulting in physiological capabilities that match the new functional demands (Allen *et al*., 1999; Pette, 2002; Pehme *et al*., 2004; Seene *et al*., 2004).

The pathophysiology of catabolic conditions (e.g., kidney failure, diabetes, burn injury, cancer, sepsis, muscle denervation, starvation, etc.) is complicated and there are numerous adverse effects. A common consequence of many of these disorders is loss of body weight and muscle mass. In healthy adults, the daily turnover of cellular proteins is the same as the amount of protein contained in 1 to 1.5 kg of muscle (Mitch & Price, 2003). This high rate of turnover in muscle is necessary to replace proteins that are incorrectly translated, that become damaged or modified, or that require rapid degradation because they regulate critical cellular functions. Consequently, a small but persistent decrease in protein synthesis or increase in proteolysis in patients with these conditions can lead to a substantial loss of muscle mass (Nader, 2005).

Skeletal muscle atrophy is a change that occurs in muscles of adult animals as a result of the conditions of disuse (e.g., immobilization, denervation, muscle unloading), aging, starvation, and a number of disease states (i.e., cachexia) (Jackman & Kandarian, 2004; McKinnell & Rudnicki, 2004) and is characterized as decreased muscle fiber cross-sectional area and protein content, reduced force production, increased insulin resistance and fatigue resistance (Jackman & Kandarian, 2004; Zhang *et al*., 2007).

After the initial physiological stimulus, in which the skeletal muscle no longer bears weight or contracts with tension, molecules involved with disuse atrophy, such as initiating triggers, signaling proteins, and affected targets, carry out the process of muscle protein loss (Jackman & Kandarian, 2004; Kandarian & Jackman, 2006).

The rapid degradation of specific proteins permits adaptation to new physiological conditions and changes in cell composition (Lecker *et al*., 1999). Protein degradation in muscle functions in maintaining normal physiological homeostasis and adapting to new homeostatic states, and is required for muscle wasting or atrophy in various pathological states (Price, 2003; Costelli *et al*., 2005; Szewczyk & Jacobson, 2005). The interplay between protein synthesis and degradation to maintain homeostasis is complex and responds to a variety of autocrine and intercellular signals from neuronal inputs, hormones, cytokines, growth factors and other regulatory molecules (Szewczyk & Jacobson, 2005).

Numerous proteolytic systems contribute to the degradation of muscle proteins. Three major proteolytic systems to skeletal muscle protein loss are: the cytosolic calcium-dependent proteases (calpains), the lysosomal proteases and the ATP-dependent ubiquitin-proteasome system (Taillandier *et al*., 1996; Jackman & Kandarian, 2004; Schakman *et al*., 2008).

2. Glucocorticoid-induced muscle atrophy

Glucocorticoid-induced muscle atrophy is characterized by fast-twitch or type II muscle fiber atrophy illustrated by decreased fiber cross-sectional area and reduced myofibrillar protein content (Kaasik *et al*., 2000; 2007; Schakman *et al*., 2008).

The catabolic effects of glucocorticoids (GCs) have been well known for many years. Either drugs used to treat several medical conditions or as endocrine hormones released in response to many stress situations, GCs may cause skeletal muscle atrophy. The resulting weakness of peripheral and respiratory muscles may have major clinical implications such as loss of quality of life, fatigue, impaired wound healing, compromised lung function, and poor immune response (Lecker *et al*., 1999; Schakman *et al*., 2008).

Various triggers and signaling proteins have been studied for roles in regulating disuse atrophy. The cascade of events that lead to disuse atrophy, beginning with reduced muscle tension and extending to the effects on protein synthesis and degradation, is not known, but several potential triggers and signaling molecules have been identified (Jackman & Kandarian, 2004).

GCs derive their name from their effect of raising the level of blood sugar (glucose). They are a class of strongly hydrophobic steroid hormones, characterized by an ability to bind to the receptor of cortisol, the most important human glucocorticoid, and trigger similar effects. GC receptors are found in the cells of almost all vertebrate tissues. GCs regulate and support a variety of vital physiological activities including cardiovascular, metabolic and homeostatic functions and are among the most frequently prescribed drugs. The traditional concept of GC action is the binding of a glucocorticoid/analog to a classical GC receptor in target cells. The resulting hormone-receptor complex next migrates to the nucleus where it regulates gene transcription both positively and negatively, ultimately leading to an appropriate change in physiological function of the target cell (Shahin, 2006). Once the

steroid hormone is bound to the GC receptor, it is able to act as a direct transcriptional regulator (Bray & Cotton, 2003).

2.1 Specific role of glucocorticoids in muscle atrophy

Glucocorticoids (GCs) are both natural (cortisol in humans, corticosterone in rodents) and synthetic (e.g., prednisolone and dexamethasone). Cortisol is a stress hormone, which stimulates gluconeogenesis, which is the formation of glucose from sources other than glucose, such as amino acids and free fatty acids. Cortisol also inhibits the use of glucose by most body cells. This can initiate protein catabolism (breakdown), thus freeing amino acids to be used to make different proteins, which may be necessary and critical in times of stress. In terms of atrophy, an increase in coritsol is related to an increased rate of protein catabolism (Viru & Viru, 2004).

GCs are lipophilic and gain access to cells by diffusion across the plasma membrane. Macromolecules like GCs that act in the cell nucleus must overcome the nuclear envelope. This barrier between cytosol and the nucleus is perforated by nuclear pore complexes (NPCs), that serve as translocation machineries (Shahin *et al*., 2005a). NPCs mediate and control transport between the cytosol and the nucleus (Rout *et al*., 2003; Shahin *et al*., 2005b; Kastrup *et al*., 2006; Shahin, 2006).

The nuclear barrier provides the cell with the opportunity to control access to its DNA (protecting vital nuclear DNA). The tightness of the nuclear barrier is therefore physiologically pivotal and any remarkable change in its structure and permeability can elicit pathophysiological changes. Nuclear barrier structure and permeability are highly responsive to hydrophobic cargos of crucial physiological and therapeutic importance, GCs (Shahin, 2006).

GC action on the nuclear barrier has been investigated on rather nonclassic target cell, namely the *Xenopus laevis* oocyte, with atomic force microscopy (AFM), fluorescence microscopy and electrical methods (Shahin *et al*., 2005b). AFM is used to image the nuclear envelope surface and estimate the hydrophobicity degree of the NPC central channel upon exposure of cells to GCs (Shahin *et al*., 2005b). The strongly hydrophobic glucocorticoids/analogs are observed to transiently remodel the structure and the permeability of the nuclear barrier (Shahin *et al*., 2005b; Shahin, 2006). Remodeling of nuclear envelope structure and permeability is prerequisite for mediating physiological actions of GCs (Shahin *et al*., 2005b). The chemical structures of the steroids are based on

natural corticosteroids, changes have been made in order to optimize therapeutic potential and minimize adverse reaction (McMaster & Ray, 2007).

Dexamethasone (DEX) is a synthetic steroid of a great therapeutic relevance that specifically binds to glucocorticoid receptors and thus triggers an intracellular signal cascade involving the cell nucleus (Shahin *et al*., 2005a).

With single molecule resolution Shahin and coworkers (2005a) observed that DEX initiated proteins first bind to NPC-free areas of the outer nuclear membrane. This caused NPCs to dilate. Then, in second step, DEX initiated proteins attached directly to NPCs and entered the dilated central channels. They concluded that DEX expose induced NPC dilation (Shahin *et al*., 2005a). GC-induced effects on the nuclear barrier may be triggered by affecting the nuclear barrier hydrophobicity. Formation of giant (nuclear) pores upon cell exposure to GCs may open unique perspectives on gene therapy, exogenously applied therapeutic macromolecules could be delivered into nuclei of non-dividing cells. The fact that GC receptors are found in the cells of almost all vertebrate tissues makes them even more attractive for gene therapeutic purposes (Shahin, 2006).

The synthetic GC dexamethasone is widely used to induce muscle proteolysis either in vivo or in cell culture. GCs are the most important and frequently used anti-inflammatory and immunosuppressive drugs in the treatment of rheumatic and other inflammatory diseases (Schäcke *et al*., 2002; Carballo-Jane *et al*., 2004; Cosío *et al*., 2005; Rosen & Miner, 2005; McMaster & Ray, 2007; Stahn *et al*., 2007).

Although the benefits of GC therapy are derived from short-term vascular changes and limited immunosuppression, prolonged or highdose GC therapy has multiple side effects (Schäke *et al*., 2002).

These multiple side effects seriously limit the value of corticosteroids in severe inflammation where the risk:benefit ratio is compromised. This has driven the need to develop novel agents with the anti-inflammatory capacity of corticosteroids but with reduced side effects (Adcock, 2004).

Many of the anti-inflammatory effects of GCs are due to inhibition of transcription factors (transrepression), whereas the endocrine and metabolic effects are mediated by binding to GC receptors in DNA (transactivation) (Cosío *et al*., 2005).

Corticosteroids exert their effects by binding to a single cytoplasmic receptor (the glucocorticoid receptor, GR) that upon activation, translocates to the nucleus where it exerts its effects (Adcock, 2004).

The response of a single cell exposed to GCs is the result of the interplay between the following three parameters: the concentration of free hormone, the relative potency of the hormone, and the ability of the cell to receive and transduce the hormonal signal (Bamberger *et al*., 1996).

Successful treatments for skeletal muscle atrophy could either block protein degradation pathways activated during atrophy or stimulate protein synthesis pathways induced during skeletal muscle hypertrophy (Glass, 2003).

2.2 Mechanisms activating proteolysis to cause muscle atrophy in catabolic conditions

Because it is difficult, if not impossible, to investigate the mechanisms responsible for disuse muscle atrophy in humans, animal models have been developed to mimic the various conditions that produce human disuse muscle atrophy (Powers *et al*., 2005).

All intracellular proteins and many extracellular proteins are continually "turning over"; i.e., they are being hydrolyzed to their consistent amino acids and replaced by new synthesis (Lecker *et al*., 1999).

Individual proteins in the nucleus and cytosol, as well as in the endoplasmatic reticulum and mitochondria, are degraded at widely different rates. For example, while most proteins in a rat liver might turn over once every one to two days, some regulatory enzymes have half-lives as short as 15 minutes. On the other hand, actin and myosin in skeletal muscle are much more stable, perhaps turning over only once every one to two weeks. Mammalian cells contain multiple proteolytic systems to carry out this degradation process and complex regulatory mechanisms to ensure that this continual proteolysis is highly selective and to prevent excessive breakdown of cell constituents. Also, the overall rates of protein synthesis and degradation in each cell must be precisely balanced, since even a small decrease in synthesis or a small acceleration of degradation, if sustained, can result in a marked loss of mass in the organism as a whole (Lecker *et al*., 1999).

Although the continual destruction of cell proteins might appear wasteful, this process has several important homeostatic functions. The rapid removal of critical regulatory proteins (e.g., transcription factors or enzymes and inhibitory factors) is essential for control of cell growth and metabolism. The rapid degradation of specific proteins permits adaptation to new physiological conditions and changes in cell composition. In all cells, protein breakdown provides an essential quality control mechanism that selectively eliminates

abnormally folded or damaged proteins that have arisen by missense or nonsense mutations, biosynthetic errors, damage by oxygen radicals, or by denaturation (Lecker *et al*., 1999).

In both fasting and acidosis, GCs at physiological concentrations appear to play a "permissive role" in the catabolic response; they are necessary, but not sufficient to account for activation of proteolysis. Besides promoting proteolysis in muscle, GCs also inhibit protein synthesis by decreasing translation of mRNAs encoding muscle proteins and supressing amino acid entry into muscle, and these coordinated actions promote the mobilization of amino acids used for gluconeogenesis (Mitch & Goldberg, 1996; Lecker *et al*., 1999). Simultaneously, in the liver, GCs induce gluconeogenic enzymes that convert amino acids into glucose. Thus, the homeostatic function of GCs in fasting involves coordinated actions in muscle to mobilize amino acids and in the liver to produce glucose from these precursors (Lecker *et al*., 1999).

This catabolic response to GCs is not seen in the fed state because of the high levels of insulin, which inhibit the activation of proteolysis. When GCs are administered in pharmacologic doses (as in the iatrogenic Cushing's syndrome), the steroids can overcome the inhibitory effect of insulin and activate muscle protein breakdown and muscle wasting even in the fed state (Lecker *et al*., 1999).

2.3 Pathways involved in protein degradation

Among multiple pathways that can cause cellular protein degradation, lysosomal proteases (cathepsins), calcium-activated proteases (calpains), and the adenosine triphosphate (ATP)-dependent ubiquitin-proteasome proteolytic pathway are considered to be the major processes responsible for the breakdown of skeletal muscle proteins in cachexia. These systems work in harmony, rather than individually, to produce muscle proteolysis (Hicke, 1999; Hasselgren *et al*., 2002; Tisdale, 2005). Cathepsines do not degrade cytosolic proteins, such as myofibrils, but their major role is to break down membrane proteins such as receptors and ion channels (Hicke, 1999) and calpains are unable to degrade actine and myosin, they seem to be involved in myofibrillar disassembly, with caplains releasing myofibrils for degradation by the ubiquitin-proteasome proteolytic pathway (Hasselgren *et al*., 2002).

The ubiquitin-proteasome pathway (UPP) plays a critical role in the adaptation of skeletal muscle to persistent decreases or increases in muscle activity (Reid, 2005). UPP is activated in numerous catabolic conditions and seems to be responsible for the bulk of

increased proteolysis and the loss of myofibrillar proteins that prevail in such instances (Attaix *et al*., 2005). Certain agents, including glucocorticoids, cytokines, and oxidative stress, are thought to be responsible for the induction of the UPP in skeletal muscle in catabolic conditions. Insulin suppresses activation of this pathway, and loss of insulin action in diabetes leads to muscle wasting (Tisdale, 2005)

In skeletal muscle, as in any mammalian tissue, protein levels are dictated by relative rates of protein synthesis and breakdown. Recent studies have shown that the ubiquitin-proteasome-dependent proteolytic pathway is mainly responsible for the breakdown of myofibrillar proteins (Price, 2003; Taillandier *et al*., 2004).

UPP functions as an essential mediator of muscle remodeling, both in atrophic states and exercise training. Pathway regulation is likely to influence processes that range from marathon training to postoperative rehabilitation, from body building to the sarcopenia of aging (Reid, 2005).

2.4 Characterization of the glucocortiocid-induced muscle atrophy: turnover of specific skeletal muscle proteins

Skeletal muscle is a complex, versatile tissue composed of a large variety of functionally diverse fiber types. The overall properties of a muscle largely result from a combination of the individual properties of its different fiber types and their proportions. Skeletal muscle fiber types, which can be delineated according to various parameters, for example, myofibrillar protein isoforms, metabolic enzyme profiles, and structural and contractile properties, are not fixed units but are capable of responding to altered functional demands and a variety of signals by changing their phenotypic profiles. The force generated by muscle is dependent on fibers size and muscle fiber type composition (Pette & Staron, 2001).

Skeletal muscle is an extremely heterogeneous tissue composed of a variety of fast and slow fiber types and subtypes. Moreover, muscle fibers are versatile entities capable of adjusting their phenotypic properties in response to altered functional demands. Muscle fibers, the contractile elements of which are actin and myosin filaments, can be classified by their metabolic, functional and morphological properties (Pette, 2002).

Myosin is the main contractile protein of striated muscle, which converts chemical energy to mechanical work. Major differences between muscle fiber types relate to their myosin complement, i.e., isoforms of myosin heavy and light chains. Contractile properties

of skeletal muscle depend on the composition of myosin heavy chain (MyHC) isoforms in the muscle. MyHC isoforms appear to represent the most appropriate markers for fiber type delineation. On this basis, pure fiber types are characterized by the expression of a single MyHC isoform, whereas hybrid fiber type expresses two or more MyHC isoforms. Hybrid fibers bridge the gap between the pure fiber types. The fiber population of skeletal muscles, thus, encompasses a continuum of pure and hybrid fiber types. Pure fiber types for example, are type IIB, type IID/X, type IIA, and type I, express MyHC IIb, MyHC IId/x, MyHC IIa and MyHC Iß respectively. The percentage of hybrid fibers increases remarkably in transforming muscles (Pette & Staron, 2001).

Under certain conditions, changes can be induced in MyHC isoform expression heading in the direction of either fast-to-slow or slow-to-fast. Increased neuromuscular activity, mechanical loading, and hypothyroidism are conditions that induce fast-to-slow transitions, whereas reduced neuromuscular activity, mechanical unloading, and hyperthyroidism cause transitions in the slow-to-fast direction (Pette & Staron, 2000).

Similar functional and structural changes in skeletal muscle in glucocorticoid and exercise myopathies have given the opportunity to speculate that exercise myopathy might be a mild form of corticosteroid myopathy (Kaasik, 2004; Lehmann *et al*., 1999). It is well known that administration of GCs induces muscle myopathy. The ultrastructural study showed the disarray of thick myofilaments in fast-twitch (FT) glycolytic fibers 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). Dissarray of thick myofilaments in myopathic muscle is a result of elevated destruction of filaments which accompanies myosin heavy chain (MyHC) degradation and causes changes in MyHC composition (Kaasik *et al*., 2000).

The changes differed in the synthesis rate of different MyHC isoforms in glucocorticoid myopathic muscle. The synthesis rate of MyHC I isoform did not change in myopathic *soleus* (SOL) muscle where this isoform makes up about 99% of MyHC I, as well as in *plantaris* (PLA) muscle where it forms about 5% of MyHC I. In myopathic muscles the synthesis rate of type II MyHC isoforms decreased. The decrease in the synthesis rate of MyHC IIb isoform and its increased degradation, the relative content of this MyHC IIb isoform decreased in myopathic skeletal muscles in which the oxidative potential is low. In myopathic muscles the relative content of MyHC IIb isoform decreased, but the relative

content of MyHC IIa and IId increased and the twitch characteristics of the skeletal muscle changed (Seene *et al*., 2003).

Protein turnover is further stimulated by changes in the pattern of muscle use. Sustained decreases or increases in physical activity cause muscle fibers to adapt, altering protein expression and fiber size. The outcome of these adaptive responses, atrophy vs. hypertrophy, reflects the net balance between protein degradation and protein synthesis. Atrophy requires that protein degradation exceed resynthesis. In muscle hypertrophy, the opposite is true (Reid, 2005).

The UPP is a primary regulator of these dynamic processes, providing a mechanism for selective degradation of regulatory and structural proteins. This pathway is constitutively active in muscle fibers and mediates both intracellular signaling and normal protein turnover. Changes in muscle use can stimulate pathway activity, altering activity-related signals and contributing to the remodeling of muscle fibers (Reid, 2005).

Although there are some similarities between the abovementioned 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 fibers (Seene, 1994; Kaasik *et al*., 2007). Aging induced sarcopenia is a result of decreased synthesis and increased degradation rate of myofibrillar proteins, which leads to the slower turnover rate of contractile proteins and to the significant decrease in hindlimb grip strength (Pehme *et al*., 2004). Dexamethasone-treatment both in the young and old group led to quite similar results, but these changes were more significant in the aging group. Both aging and dexamethasone-induced sarcopenic muscles have diminished regenerative capacity (Kaasik *et al*., 2007).

3. Hypertrophy and atrophy – structural and functional aspects

It is well established that skeletal muscle can adapt to the variable functional requirements through a quantitative mechanism based on changes in muscle mass and fiber size, and a qualitative mechanism based on a change in fiber type distribution (Harridge *et al*., 1996).

Atrophy and hypertrophy are two opposite conditions that can be found in muscles. Atrophy is characterized by a loss of the muscle mass and usually involves a decrease in the size or cross-sectional area (CSA) (Jackman & Kandarian, 2004).

To maintain homeostasis, the biological response of the human body generates a dynamic balance between synthetic and degradative processes (Mitch & Goldberg, 1996; Lecker *et al*., 1999) for both atrophic and hypertrophic muscles (Boonyarom & Inui, 2006). This dynamic balance occurs in response to any stimuli (Hoffmann & Nader, 2004) due to processes that promote muscle growth via increased protein content (Glass, 2003; Boonyarom & Inui, 2006).

Muscle growth and adaptation is a complex and integrative process (Russell *et al*., 2000). Moreover, hypertrophy can result either from increased protein production, decreased protein breakdown, or a combination of both of these aspects of protein turnover (Boonyarom & Inui, 2006).

Through exercise, the muscular work done against a progressively challenging overload leads to increases in muscle mass and CSA, referred to as hypertrophy (Goldspink, 1999).

The muscle is able to adapt by increasing the size and amount of contractile proteins, which comprise the myofibrils within each muscle fiber, leading to an increase in the size of the individual muscle fibers and their consequent force production (Russell *et al*., 2000).

Animal models have been developed to mimic the various conditions that produce human disuse muscle atrophy and there are different animal models, which are clearly linked to hypertrophy responses under diverse experimental conditions (e.g., models of resistance exercise and chronic functional overload) (Adams & Haddad, 1996; Adams *et al*., 1999; Goldspink, 1999; Powers *et al*., 2005; Garma *et al*., 2007). In the case of chronic overload the rats were overloaded via the unilateral ablation or partial surgical removal of synergist muscle, this is also called as compensatory muscle hypertrophy (Seiden, 1976; Ianuzzo & Chen, 1977).

Studies have shown that these different conditions causing atrophy or hypertrophy influence skeletal muscle fibers differently. GC-induced atrophy affects mainly fast muscle fibers by decreasing their CSA (Edgerton *et al*., 1995). Hypertrophy, however, due to synergist ablation or tenotomy affects both fast and slow muscle fibers by increasing their CSA (Yamauchi *et al*., 1996).

Overload-induced hypertrophy is a complex event, but the research in this area supports a two-stage model of muscle adaptation to overload: (1) during regulation at the onset of hypertrophy, muscle protein synthesis increases during overload-induced hypertrophy (Goldspink, 1977; Wong & Booth, 1990); and (2) during regulation at later stages of hypertrophy, myofibrillar protein mRNA levels increase later from overload-

induced enlargement (Booth *et al*., 1998). The increase in translational capacity is indicated by increased numbers of ribosomes, which leads to protein expression and protein synthesis (Nader *et al*., 2002). The increased mRNA template can be achieved by increasing the transcription rate of the given gene and/or the addition of a satellite cell derived nuclei. In the case of hypertrophy, numerous studies have reported that satellite cells proliferate and then fuse with existing myofibers as fully differentiated skeletal muscles adapt to increased loading (Carson & Alway, 1996) and that this process appears to be obligatory for the development of compensatory hypertrophy (Rosenblatt *et al*., 1994; Phelan & Gonyea, 1997; Adams *et al*., 1999; Hawke & Garry, 2001).

The physiological features of skeletal muscle atrophy include a marked tendency of slow muscle fibers to take on fast characteristics, increased fatiguability and myofibrillar protein loss due to decreased synthesis and increased degradation. Given the extent of remodeling that occurs, it is clear that atrophy is not simply a degradative process. For example, genes defining fast-twitch, glycolytic phenotypes and those involved in proteolytic processes (Taillandier *et al*., 1996) are actually upregulated in the face of this global protein loss (Stevenson *et al*., 2003). So, while certain genes are downregulated and preferentially degraded, others are spared from destruction or even transcriptionally upregulated (Stevenson *et al*., 2003).

The causes of muscle atrophy are from several sources, such as neuromuscular diseases, immobilization and denervated conditions (Mitch & Goldberg, 1996 Jackman & Kandarian, 2004; Boonyarom & Inui, 2006). The general appearance indicates a noticeable reduction in the muscle CSA when the muscle is in an atrophic condition (Mujika & Padilla, 2001; Widrick *et al*., 2001; Hudson & Franklin, 2002). Disappearance of myonuclear is one of the pathological signs of muscle atrophy (Allen *et al*., 1996; Hikida *et al*., 1997; Edgerton *et al*., 2002). Several groups of investigators suggested that the quantitative loss of myonuclei during muscle atrophy is not always proportional to the decrease of muscle fiber CSA, but to a smaller myonuclear domain size (Allen *et al*., 1996; 1997).

The increase and/or loss in the number of myonuclei result in alterations of metabolic activity, which in turn leads to an increase or decrease in protein content and cell size.

II AIMS OF THE STUDY

The study was undertaken in order to investigate the effect of glucocorticoid-treatment on skeletal muscle cell size and shape in muscles with different twitch characteristics. The additional aim was to study the possibilities of recovery of atrophied skeletal muscles using chronic overload.

The specific aims of the study were as follows:

1. To examine the effect of glucocorticoid-treatment on skeletal muscle with different twitch characteristics.

2. To clarify qualitative changes in skeletal muscle tissue, due to the effect of glucocorticoid-treatment.

3. To clarify qualitative morphological changes in skeletal muscle.

4. To study the effect of functional overload on skeletal muscle contractile proteins in recovery from glucocorticoid-induced atrophy.

III MATERIALS AND METHODS

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

1. Animals and experimental design

The used animals (National Laboratory Animal Centre, Kuopio, Finland) were 16-17 weeks old (at the beginning of the experiment) male rats of the Wistar strain. All the animals were housed in identical environmental conditions in polycarbonate type III cages, at 21 ºC, two per cage at 12/12h light/dark period. They received diet (*SDS-RM1(C) 3/8, Witham, Essex, England*) and water *ad libitum*. The rats were randomly divided into groups: the control (C) (n=8), dexamethasone-treated (DEX) group (n=8), dexamethasone-treated and compensatory hypertrophy (DEX+CH) group (n=8).

2. Induction of muscle atrophy

Dexamethasone (DEX) (*Dexafort, Intervet, Holland*) was diluted to 200 μg/ml with 0.15 M NaCl and administered intraperitoneally daily 100 μg/100 g bw during 7 days. The control animals received appropriate amounts of 0.15 M NaCl.

3. Induction of muscle hypertrophy

After 7-days administration of DEX compensatory hypertrophy was applied by synergist tenotomy, which lasted for 10 days. Before synergist tenotomy the animals were anesthetized with ketamin (*Calysol, Gedeon Richter A.O., Budapest, Hungary*) 2.5 mg/100g bw and diazepam (*Lab Renaudin*, *France*) 2.5 mg/100g bw. The *soleus* (SOL) and *plantaris* (PLA) muscles were overloaded by unilateral removal of its synergist, the *gastrocnemius* (GAST) muscle. Muscles of the contralateral leg served as a control.

4. Tissue collection

Twenty-four hours after 7-days DEX treatment (DEX group) and after 10-days tenotomy (DEX+CH group) the animals were anesthetized with ketamin (*Calysol, Gedeon Richter A.O., Budapest, Hungary*) 2.5 mg/100g bw and diazepam (*Lab Renaudin*, *France*) 2.5 mg/100g bw and sacrificed.

SOL and PLA muscles were quickly removed, cleared of external connective tissue, weighed, frozen in liquid nitrogen, and stored at –80 ºC until analyzed further.

5. Histological assessment of muscle atrophy

Muscle specimens for histological analysis were obtained from muscle's midbelly by cutting transversely across the center of the muscle and fixed in embedding medium (*Tissue-Tek, O.C.T. Compound, USA*) and quickly frozen in isopentane cooled with liquid nitrogen. Serial cross-sections were obtained from each muscle midbelly (10 μm thickness) by a cryostat microtome (*Cryo-Cut, American Optical Company*) at –20 ºC, and attached to glass slides.

To assess the cellular architecture of SOL and PLA muscle, the cross-sections were stained with Mayer's Hematoxylin-Eosin (H&E). It is a representative histological method that emphasizes the morphological composition of cells and tissues. With Mayer's H&E stain the sarcolemmal nuclei stained blue, the muscle fibers pink and the connective tissue a lighter pink.

Stained sections were visualized with an Olympus BX 41 equipped with video capture device Color View IIIu (*Olympus, Japan*), using a 20X, a 40X and a 100X objective. A random sample of 100-150 fibers from each muscle was analyzed using Color View Soft Imaging System Software (*Olympus Biosystems, Germany*).

6. Immunohistochemistry and histochemistry

In both muscles, histochemically defined different types of fibers reacted with monoclonal antibodies (*Novocastra Laboratories Ltd, Newcastle upon Tyne, UK*) against MyHC fast (NCL – MHC_f) and slow isoforms (NCL – MHC_s). In brief, sections were preincubated in a blocking solution of stock goat serum. The primary monoclonal antibody was then applied and allowed to incubate overnight in a humid chamber at 4 °C. The second day the sections were washed and then reacted with a secondary antibody. Sections were again washed and reacted in avidin-biotin peroxidase complex reagent. Diaminobenzidine tetrahydrochloride was used as chromogen to localize peroxidase.

Additional cross-sections were stained for mATPase activity, following alkaline (pH 10.3) preincubation by Brooke & Kaiser (1970). Stained sections were visualized as described above.

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). myofibrillar protein was extracted with three volumes 100 mM sodium pyrophosphate, 5 mM EGTA, 1 mM dithiothreitol (pH 8.5) diluted with glycerol 1:1 (vol/vol) and were stored at –80 ºC until analysis.

8. Determination of whole muscle MyHC isoform composition

MyHC were used as molecular marker to assess the fiber type composition of each mucle, and their expression was determined by gel electrophoresis. MyHC isoforms were separated by 7.2% SDS-PAGE using 0.75 mm thick gel. Myofibrils 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. Electrophoresis lasted for 24 h at 120 V (Hämäläinen & Pette, 1996). Gels were silver-stained by the method of Oakley *et al*. (1980). Protein bands were identified by their known molecular weights and marker proteins. Protein isoform bands were analyzed densitometrically by Image Master® 1 D program, Version 4.0 (*Amersham Pharmacia Biotech, UK*) and the percentage distribution of MyHC various isoforms was evaluated.

9. Statistical analysis

Means and standard errors of the means were calculated from the individual values by the standard procedures of Excel. The two-tailed independent *t*-test was used for comparison of two populations. Differences were considered significant at $p<0.05$.

IV RESULTS

1. *Changes in rats body weights during dexamethasone-treatment and compensatory hypertrophy*

The mean body weight responses resulting from dexamethasone-treatment (DEX) and compensatory hypertrophy (CH) are represented in Figure 1. There were significant decreases in the body weights of rats during 7-days DEX-treatment (Fig. 1, $p<0.01$). Applying synergist elimination by tenotomy after the final administration of DEX caused the increase in the body weights of animals (Fig. 1, $p<0.05$). The Figure 1 illustrates the decrease in body weight over the 7-days DEX-treatment period also there is a little increase over 10-days CH. Thus, by the end of the 7-days drug treatment, the body weights of the rats in DEX-treatment group were 20% less than those of the rats in the control group.

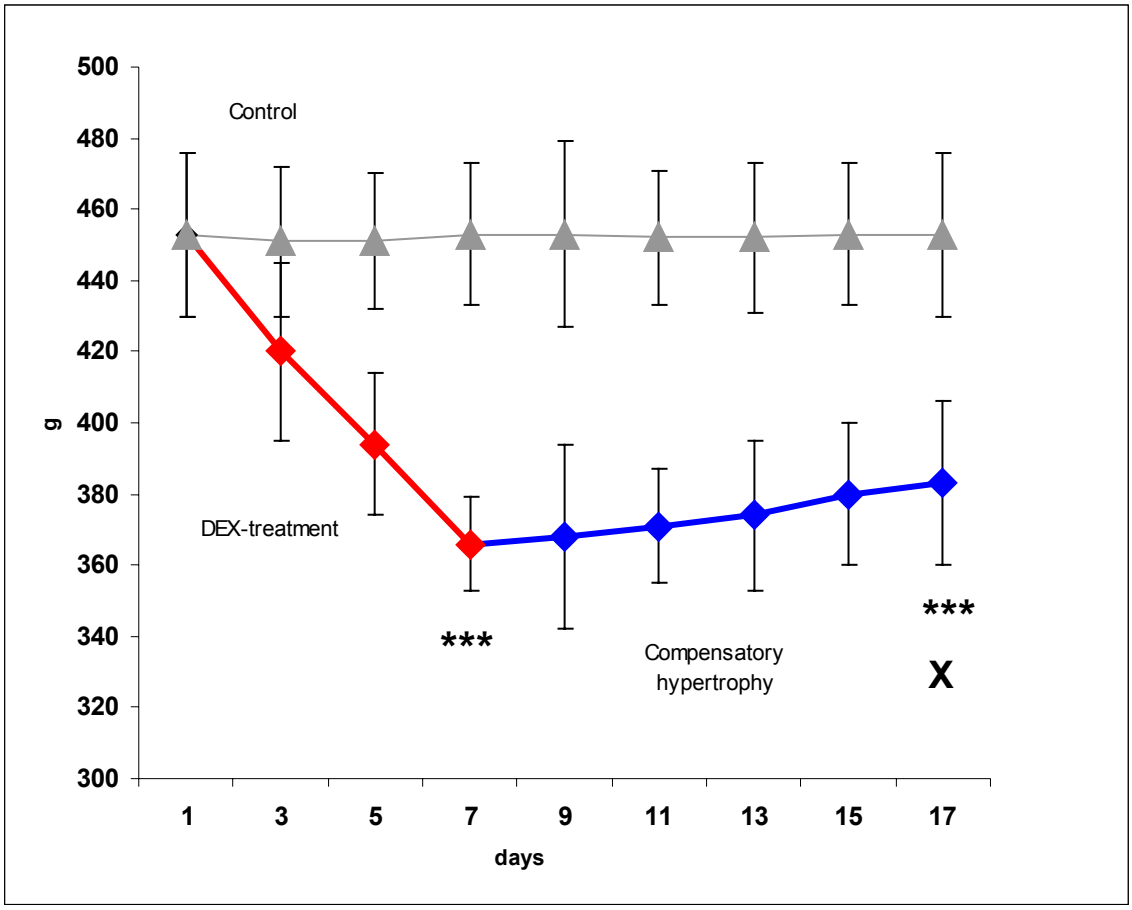

Figure 1. Changes in rats body weights during dexamethasone-treatment (DEX) followed by elimination of synergist function (DEX+CH). x±m; ***-$p<0.01$ compared with control group, X-$p<0.05$ compared with DEX group, n=8.

2. *The effects of dexamethasone-treatment and compensatory hypertrophy on muscle weights*

Similarly to changes in the body weights, SOL and PLA weights in DEX group were lower than in the control and DEX+CH group (Fig. 2). Changes in the weight of SOL and PLA compared to their respective controls showed that the slow-twitch (ST) muscle (SOL) was less affected by the DEX. However, controversal changes were found in fast-twitch (FT) skeletal muscle (PLA) (Fig. 2, $p<0.01$).

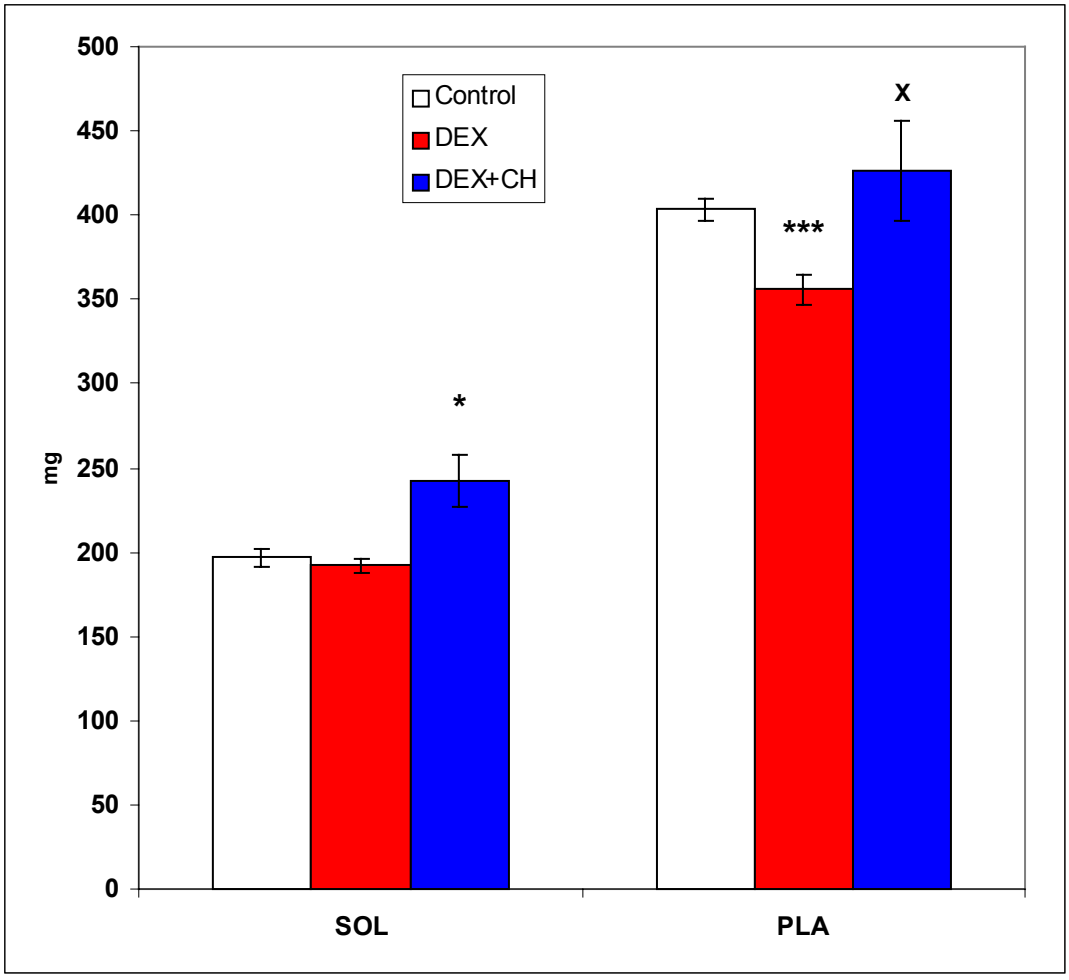

Figure 2. Changes in *soleus* (SOL) and *plantaris* (PLA) muscle weights during dexamethasone-treatment (DEX) followed by elimination of synergist function (DEX+CH). x±m; *-$p<0.05$ compared with control group, ***-$p<0.01$ compared with control group, X-$p<0.05$ compared with DEX group, n=8.

Applying overload in conditions of atrophy caused remarkable increase in weights of both slow-twitch (SOL) and fast-twitch (PLA) muscles, whereas in case of PLA muscle weight fully recovered from atrophy and exceeded the control value.

3. Histological characteristics of overloaded atrophic muscle tissue

Routine hematoxylin and eosin staining was used to analyze the complexity of skeletal muscle tissue during development of glucocorticoid-caused atrophy. Our data showed the disorders in structural components of tissue. In detail, endomysial connective tissue elements between muscle cells increased with the progression of atrophy (indicating the increased fibroblast activity). Some myonuclei, showing destructive processes in muscle cells, were also observed in inter-cellular space (Fig. 3).

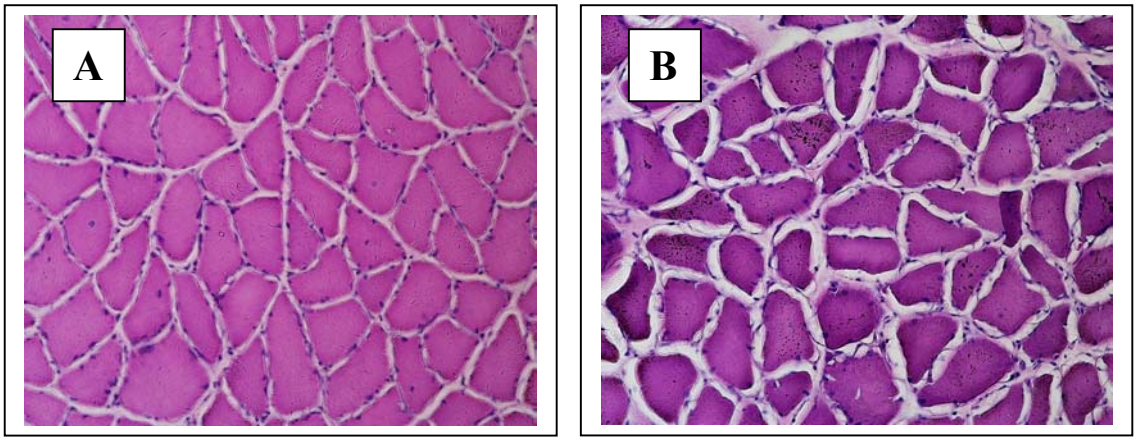

Figure 3. Hematoxylin and eosin histochemical stains of control (A) and atrophied (B) *plantaris* muscle. Disorganisation of skeletal muscle structure, magnification 20X.

The architecture of muscle tissue became disorganized, and fatty and fibrous connective tissue occupied the space left by degenerative fibers. There may be defects of plasma membrane associated with pathological alterations in the underlying fiber region in a proportion of non-necrotic fibers in atrophy. The plasma membrane lesions led to segmental fiber necrosis followed by regeneration (Fig. 3, Fig. 4 and Fig. 6).

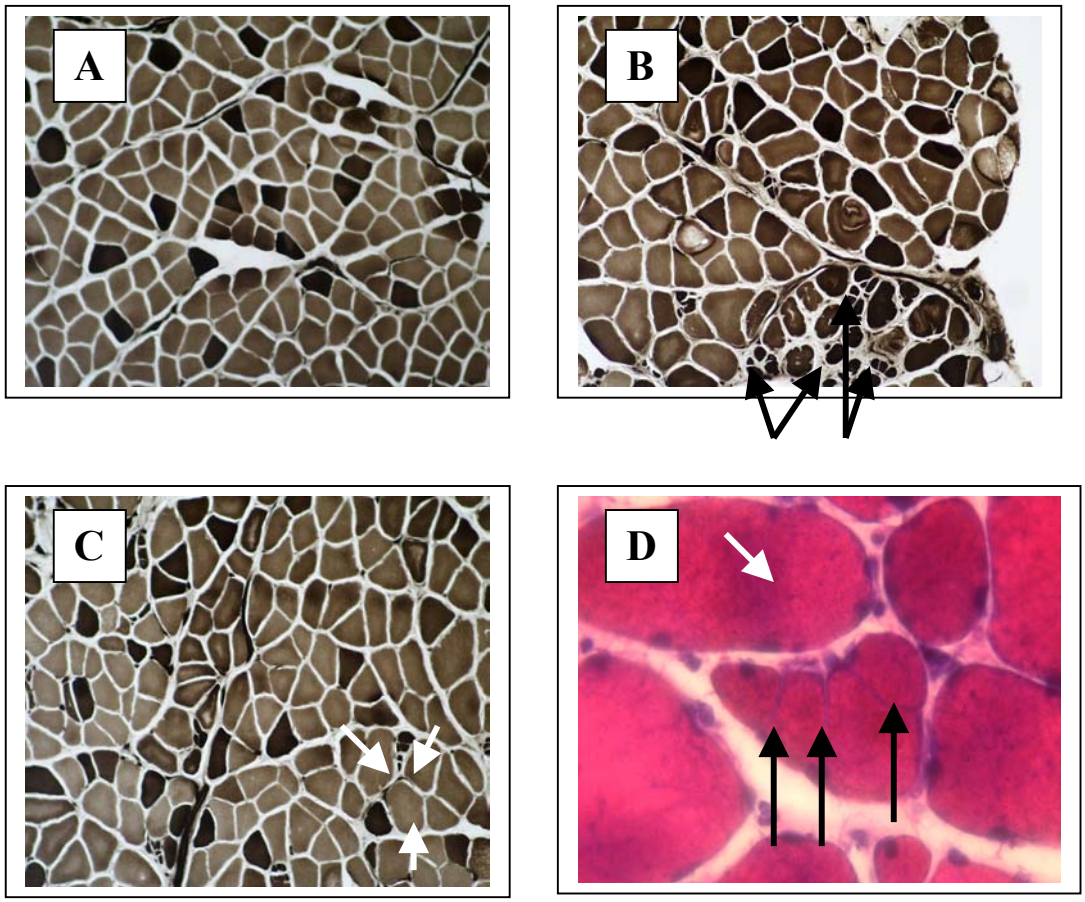

Figure 4. Histograms of a control (A) and atrophied *soleus* muscle (B, C, D). Immunohistochemical staining, stained fibers – reaction against fast MyHC isoforms. Small atrophied fibers (B - black arrows), hypertrophied fibers around atrophied fibers (C – white arrows, D – white arrow), longitudinal splitting of myofibers (D – black arrows), hematoxylin and eosin staining, magnification 20X (A, B, C) and 100X (D).

Many atrophying and splitting fibers were observed after DEX-treatment. As shown in Figure 4 and in Figure 5 only FT muscle fibers are affected by GCs. Degenerative processes of muscle cells cause changes in opposite directions in neighboring fibers resulting in atrophy caused by hypertrophy of ST fibers (Fig. 4 C, D).

Figure 5. Atrophied *soleus* muscle stained with myosin ATPase (pH 10.3). Dark fibers are fast-twitch fibers. Atrophied fibers vary abnormally in diameter and show abnormal contours (white asterisks), magnification 40X.

Our data showed abnormalities in histological characteristics in atrophied muscle fibers. We found that alongside with the development of atrophy there were remarkable changes in shape and contours of muscle cells (Fig. 5). Atrophied muscle fibers became more angular and smaller.

Atrophied fibers showed a tendency to necrosis, as many were splitting and degenerative muscle fibers were seen in cross-sectional samples of studied muscles (Fig. 6).

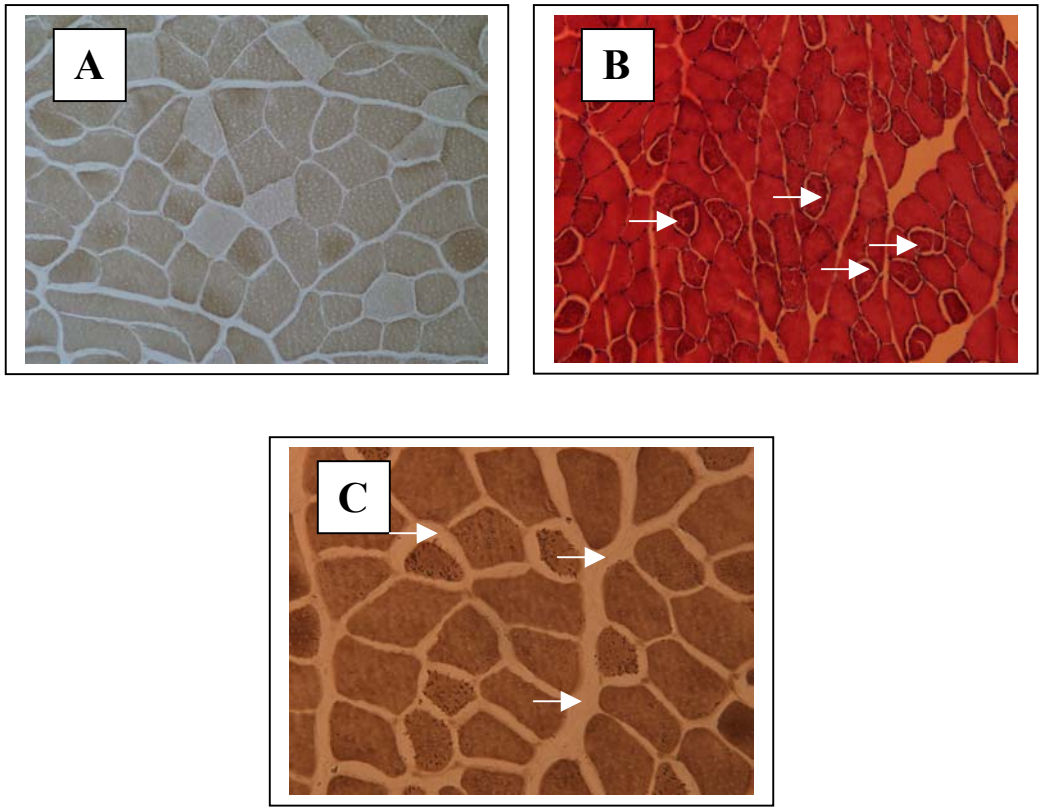

Figure 6. Splitting of myofibers (*plantaris* muscle) (B – white arrows) and connective tissue becomes more prominent (C – white arrows). A – control tissue, magnification 40X (A, C) and 20X (B).

4. The effect of overload on the distribution of MyHC isoforms and histological characteristics in atrophied muscles

MyHC isoforms were used as molecular marker to assess the fiber type composition of each muscle and their expression was determined by gel electrophoresis. MyHC isoform composition was determined in two hindlimb muscles (SOL and PLA). Examples of electrophoretic separation of MyHC isoforms in SOL and PLA muscles of the DEX and DEX+CH groups of rats are shown in Figure 7. DEX+CH induced a dramatic shift of MyHC distribution in ST muscle (SOL) towards fast MyHC IIa and MyHC IId with a concomitant decrease of MyHC I in comparison with control group.

DEX+CH had the similar effect on FT muscle (PLA) inducing a significant shift towards fast MyHC isoforms with increase in MyHC IIb and MyHC IId with a reduction of MyHC IIa in comparison with control group. DEX+CH induced an overall shift from slow-to-fast MyHC isoforms in comparison with control group in both skeletal muscles (Fig. 7).

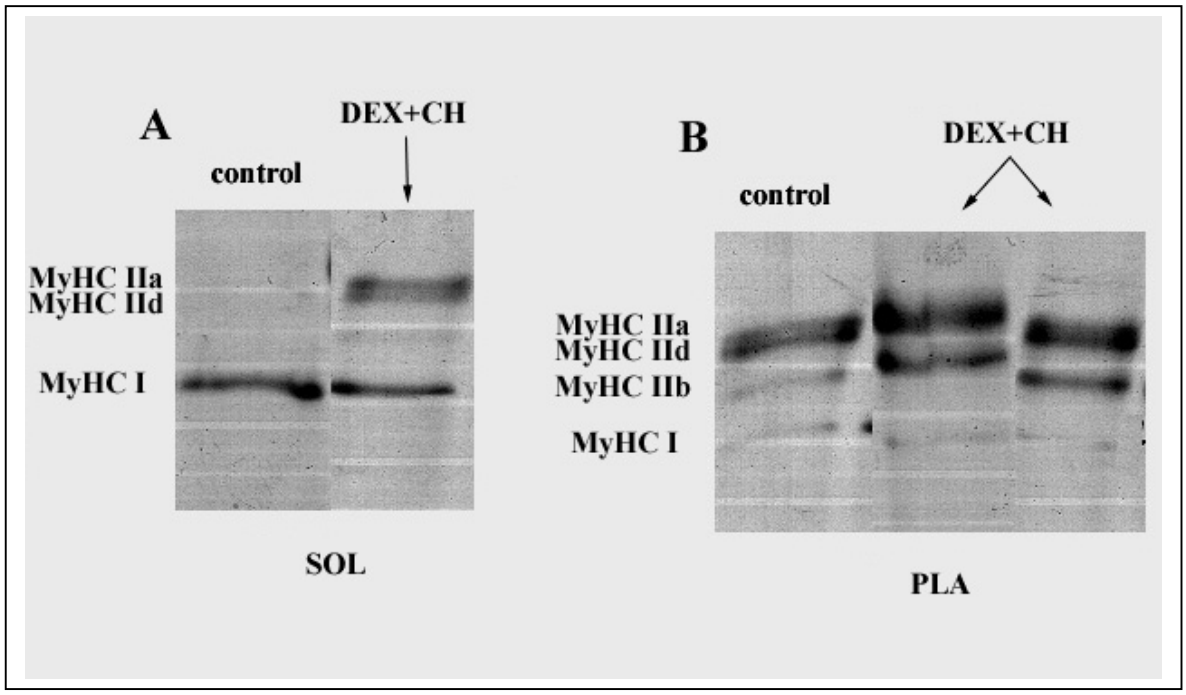

Figure 7. Electrophoretic separation of MyHC isoforms in *soleus* (A) and in *plantaris* (B) muscle. Data demonstrate qualitative remodelling of the isoformal profile during overloading the atrophied muscles. Note expression of fast isoforms in *soleus* muscle (MyHC IIa and MyHC IId) and pronounced expression of fastest isoform (MyHC IIb) in *plantaris* muscle.

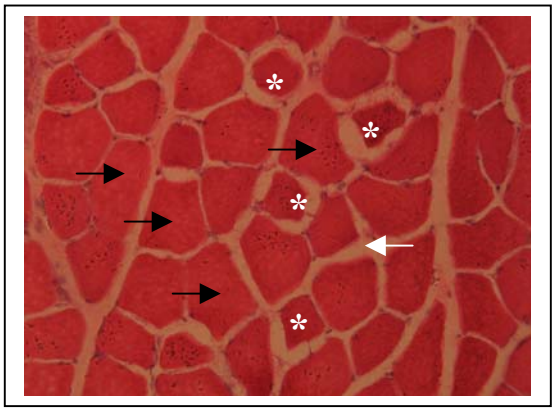

Figure 8. Hematoxylin and eosin histochemical stains from *soleus* muscle. Hypertrophied fibers (black arrows) and remaining atrophied fibers (white asterisks) during overloading the atrophied muscle. Connective tissue between muscle cells (white arrow), magnification 40X.

Overloading muscles by elimination of synergist function caused non-atrophied muscle fibers to hypertrophy but did not cause significant shifts towards regeneration and repair of atrophic fibers. Affected myofibers remained smaller in diameter and surrounded by larger amount of connective tissue (Fig. 8).

V DISCUSSION

Skeletal muscle has evolved as a tissue whose primary function is to move objects against the force of gravity, and there is a close relationship between the size and metabolism of this tissue and gravitational force. When a heavy object is moved repeatedly, the muscle cells enlarge by hypertrophy, whereas a reduction in muscle tension or use, as occurs in bedridden patients and astronauts in space, leads to rapid skeletal muscle wasting (Edgerton *et al*., 1995; 2002; Allen *et al*., 1996; Booth *et al*., 1998).

The pathophysiology of catabolic conditions (e.g. kidney failure, diabetes, burn injury, cancer, sepsis, muscle denervation, starvation, etc.) is complicated and there are numerous adverse effects. A common consequence of many of these disorders is loss of body weight and muscle mass. In healthy adults, muscle proteins turn over at a rate that roughly approximates 1–1.5 kg of muscle mass per day (Mitch & Price, 2003). This high rate of turnover in muscle is necessary to replace proteins that are incorrectly translated, that become damaged or modified, or that require rapid degradation because they regulate critical cellular functions. Consequently, a small but persistent decrease in protein synthesis or increase in proteolysis in patients with these conditions can lead to a substantial loss of muscle mass. This is important because loss of lean body mass is associated with increased morbidity and mortality.

A common feature of Cushing's syndrome and steroid therapy is the development of muscle atrophy (steroid myopathy) as a symptom or side effect, respectively (Schäcke *et al*., 2002). This fact represents a clear proof for a direct or indirect catabolic effect of excess GCs on skeletal muscle. The biological significance of such effect lies in the altered regulation of metabolism under conditions of stress. Thus, during fasting periods, infection or cancer, when the energetic and substrate requirements of the organism are increased, muscle tissue, constituting 40% of total body mass, is a rich source of amino acids. They can be mobilized and serve as substrates for energy generation, gluconeogenesis and protein synthesis. On the other hand, inhibition of glucose utilization in muscle in response to GCs increases the availability for other tissues, e.g., brain, immune system and tumor (Vegiopoulos & Herzig, 2007).

Skeletal muscle metabolism and the balance between hypertrophy and atrophy are controlled by the action of counter-regulatory anabolic and catabolic signals. In general,

contractile activity, nutritional status and the presence of disease/injury determine the type and intensity of signals affecting muscle tissue (Vegiopoulos & Herzig, 2007). The rapid degradation of specific proteins permits adaptation to new physiological conditions and is essential for changes in cell growth and metabolism (Lecker *et al*., 1999).

Extended periods of skeletal muscle inactivity (e.g., chronic bed rest, space flight, or limb immobilization) are associated with a loss of muscle protein and reduced force generating capacity (Allen *et al*., 1996). Knowledge of what regulate muscle atrophy is important in developing and anti-catabolic strategy to prevent or retard protein loss and maintain physiological function of skeletal muscle. Ongoing research has led to an improved understanding of those factors that contribute to muscle atrophy during pathologies that promote muscle atrophy or wasting. In particular, a large volume of research indicates that apoptosis and proteolytic pathways are important contributors to numerous cellular signaling that modulate muscle atrophy during prolonged inactivity. Hence, reduced activity of skeletal muscle results in a net loss of total muscle protein due to a both a decrease in protein synthesis and an increase in proteolysis (Goldspink, 1977; Booth *et al*., 1998; Goldspink, 1999).

Due to the complexities involved in investigating the mechanisms responsible for muscle atrophy in humans, numerous experimental animal models have evolved to stimulate conditions that lead to muscle atrophy (Edgerton *et al*., 2002; Hudson & Franklin, 2002; Mujika & Padilla, 2001; Widrick *et al*., 2001; Hikida *et al*., 1997) or hypertrophy (Seiden, 1976; Ianuzzo & Chen, 1977; Adams & Haddad, 1996; Yamauchi *et al*., 1996; Adams *et al*., 1999; Goldspink, 1999; Powers *et al*., 2005; Garma *et al*., 2007).

We used DEX-treated animals to produce catabolism because it is well-characterized model that produces protein breakdown in skeletal muscle (Hickson & Marone, 1993; Kaasik *et al*., 2000; 2007; Seene *et al*., 2003).

The results obtained showed that rat muscles changed their size and MyHC isoform composition in response to both dexamethasone administration and compensatory hypertrophy (CH). GCs and CH had opposite effects on body weights and muscle mass of rats. As expected, the DEX decreased body weights, and the weight of FT muscle (PLA), but did not elicit any atrophic changes in ST muscle mass. Interestingly, the response varied among the muscles analyzed, whereas the FT muscle (PLA) was the most responsive to both treatments and showed clear variations of MyHC isoform composition and the ST muscle (SOL) appeared to be less responsive to these treatments. The distinct responsiveness might depend on fiber type composition, as SOL is mainly composed of ST fibers, whereas DEX-

treatment affected more FT muscle (PLA). The higher responsiveness of fast fibers to GCs might be explained by the protective action of activity from GC-induced atrophy, slow and fast IIA fibers are more active than fast IIX and IIB fibers.

Mammalian skeletal muscle fibers display a great adaptive potential. This potential results from the ability of muscle fibers to adjust their molecular, functional, and metabolic properties in response to altered functional demands, such as changes in neuromuscular activity or mechanical loading. Adaptive changes in the expression of myofibrillar and other protein isoforms result in fiber type transitions. These transitions occur in a sequential order and encompass a spectrum of pure and hybrid fibers. Depending on the quality, intensity, and duration of the alterations in functional demand, muscle fibers may undergo functional transitions in the direction of slow or fast (Pette, 2002).

There is a relationship between the functional characteristics of a muscle and the types of MyHC isoforms that are expressed in the muscle fibers. It is also clear that MyHC isforms represent the best possible marker of fiber type transition. Muscle fibers have the unique ability to undergo changes in MyHC phenotype as an adaptive response to altered loading states. The resulting adaptive changes at the molecular level affect the functional elements involved in energy metabolism and/or the protein composition of the myofibrillar apparatus. Thus, MyHC composition of muscle fibers is the main determinant of important functional properties such as the velocity of contraction, fatigue resistance and mechanical efficiency (Pette & Staron, 2000; 2001).

One of the general aims of the study was to investigate the distribution of various MyHC isoforms in the SOL and PLA muscles in relation with several morphological variables of muscle fibers. It has been shown that the development of muscle hypertrophy is accompanied with the changes of isoformal pattern towards slow isoforms (Yamauchi *et al*., 1996; Roy *et al*., 1999; Pehme *et al*., 2004). On the other hand GCs induce the decrease in the proportion of the fast isoforms of contractile proteins (Seene *et al*., 2003; Kaasik *et al*., 2007) In the response to increased loading is evident in the expression of MyHC proteins, the shift to faster MyHC expression in DEX+CH muscles represents a compensatory adaptation most likely stimulated by the ability of these muscles to increase their mass or CSA. First, the initial ability of the DEX+CH muscles to respond appropriately to the increase in loading state indicates that the cellular systems associated with anabolic processes (e.g., increased translation and transcription) were probably not damaged by the DEX administration.

Muscle tissue is regarded as having an excellent capacity to repair itself. The ability of any striated muscle to regenerate is related to the extent of the tissue necrosis, the

prevention of the innervation and blood supply to the area, and the degree of intactness of the architecture of the muscle. Probably a very important additional factor is the nature of the initiating atrophy process. It appears that the size of the area to be repaired and the degree to which muscle architecture is destroyed are the most important factors governing collagen proliferation. The larger the devastated area, the more massive is the proliferation of collagen. Some small fibers represent the products of degeneration of muscle fibers as well as ineffective attempts at regeneration. The most important limiting factor of the regeneration might be the loss of myonuclei and lower activity of satellite cells occurred during muscle atrophy. On the other hand the existing population of myonuclei is capable to express synthesis of different isoforms of muscle proteins due to plasticity of regulation of gene expression. When two muscle fibers are in close apposition and occupy the space of one, it can usually be assumed that they have undergone splitting or branching (Fig. 4 and 6). This is a frequent alteration in primary diseases of muscle, and it is particularly conspicuous to atrophy. This process is best observed in cross-sections, where the muscle fibers are seen in close proximity and nestled within the same endomysial connective tissue enclosure, like the pieces of jigsaw puzzle. The angulated fibers, as seen in Figure 5 are small in diameter and has concave sides. Fibers such as these may occur singly or in groups in certain stages of atrophy.

Unfortunately, the methods used in this study do not allow for the differentiation of responses that would be purely catabolic and/or anabolic from those that were promoting attempts at cellular level. For example, decreased or increased protein production within myofibers is probably reflected by the decrease or increase in total RNA as less or more ribosomes are produced to meet the demand for translation. Several studies report specific phenotypic differences in the myoblast populations that contribute to the development of fast vs. slow mammalian muscles, resulting in different adaptive ranges for slow vs. fast muscles (Rosenblatt *et al*., 1996). Since a multitude of signals (i.e. developmental, electrical, hormonal, loading etc.) can alter the expression of MyHC isoforms in a fiber, it is likely that the expression of a given phenotype by a fiber results from the integration of all of these signals at the nuclear level. Thus, alterations of one or more of these signals may present the fiber with appropriate information for expression of multiple MyHC isoforms simultaneously. In other words, each individual fiber interprets all of the signals it receives and responds by expressing a given MyHC or combination of MyHCs (Talmadge *et al*., 1999).

We found that the SOL was capable of expressing fast isoforms (a muscle that is normally predominantly slow). In summary, DEX+CH resulted in a gradual loss of MyHC I and sequential increases in the levels of MyHC IIa and MyHC IId in ST muscle (SOL), similar changes were also in FT muscle (PLA), where the level of MyHC IIb and MyHC IId increased, but not MyHC IIa in comparison with control group (Fig. 7).

It appears that PLA muscle mass is more responsive than SOL mass to both treatments DEX and CH caused by synergist elimination, differences in recruiment and loading preclude the interpretation that FT fibers have greater inheret plasticity than ST. For instance, although the SOL is primarily comprised of ST fibers and has a smaller growth response than the PLA muscle, the magnitude of cell growth appears to be greater in the FT fibers than in the ST fibers of PLA muscle (Aru, 2004).

It is tempting to combine the data from slow-to-fast transforming muscles in a general scheme of reversible transitions in MyHC isoform expression namely MyHC Iß ↔ MyHC IIa ↔ MyHC IId/x ↔ MyHC IIb (Pette & Staron, 2000). According to this scheme, fiber-type transitions occur in a stepwise manner, encompassing up- and downregulations of MyHC isoforms in a gradual sequence. Moreover, depending on their position in the MyHC isoform spectrum, some fibers have the ability to transform in either direction. Fiber-type-specific options in the fast or slow direction are species-specific and muscle-specific differences to altered functional demands (Pette, 2002). Thus, the developmental origin or state of the muscle may place limits and/or restrictions as to the types of MyHC isoforms that can be expressed (Talmadge *et al*., 1999).

The effect of increasing muscle activity may vary according to the initial status of the muscle concerned, i.e. atrophied, normal and hypertrophied muscle may all respond differently. Some data indicate that normal daily activation has a coordinating effect on directing the expression of phenotypic proteins within individual muscle fibers. It is possible that some threshold level of activations is required to coordinate the expression of phenotypic proteins among the many myonuclei within an indivdual fiber (Talmadge *et al*., 1999).

In summary, the results of this study demonstrate that CH, which is caused by elimination of synergist muscle, essentially prevents the further development of atrophy in rodent skeletal muscles. Short-lasting DEX-treatment combined with CH does not appear to induce siginificant damage to myofibers or the intrinsic mechanisms necessary for them to adapt to increased loading. The results of this study tend to support the hypothesis that the

mechanisms by which myofibers adapt to different treatments appear to include several myogenic mechanisms.

The results obtained in this study showed that the combined treatment with DEX and CH tended to minimize the variations of any parameter, for example body weight growth was stimulated by CH, inhibited by DEX (Fig. 1). In SOL and in PLA muscles, the expression of fast MyHC isoforms was also stimulated by the combined treatment (Fig. 7). Our results show that the combined treatment compensates the effects of DEX, although to different extent in different muscles.

Although muscle response to altered patterns of activity has been extensively studied, there are still many areas of uncertainty. However, there are still few guidelines as to the optimal load, frequency or repetitions needed to prevent impairment, maintain normal function and/or improve performance. One of the reason for this is the individuality of responses to different treatments. The gene-environment interaction is complex, but it is possible that genotype may determine this phenotypic response. Understanding the cellular basis of the GC-induced skeletal muscle atrophy will contribute to the rational development of therapeutic interventions and therefore minimize the debilitating effects of the muscle atrophic response to CGs.

VI CONCLUSIONS

1. Glucocorticoid-treatment caused well-pronounced atrophy in fast-twitch skeletal muscle.

2. Administration of glucocorticoids evoked disorganization of skeletal muscle tissue and the increase in connective tissue between muscle cells.

3. Glucocorticoid-treatment caused well-pronounced atrophy in fast-twitch skeletal muscle cells accompanied with alterations in morphological characteristics of muscle cells: abnormalities in contour and shape, destruction of cells, longitudinal splitting of cells.

4. Overloading of atrophied skeletal muscle induced changes in the expression of MyHC isoforms. Fast isoforms were expressed in slow-twitch skeletal muscle and more intensively expressed in fast-twitch skeletal muscle.

5. Overloading of atrophied skeletal muscles did not bring about significant repair of the damaged tissue structures.

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VIII SUMMARY IN ESTONIAN

Seosed glükokortikoidse müopaatia ja funktsionaalse ülekoormuse vahel: muutused lihasraku morfoloogilistes karakteristikutes ja lihasvalkude ekspressioonis

Skeletilihast iseloomustatakse kui äärmiselt plastilist kudet, mis on suurtes piirides võimeline adapteeruma muutunud keskkonna tingimuste ning funktsionaalse aktiivsuse/inaktiivsuse eripäradega.

Erisuunaliste kohanemisprotsesside (hüpertroofia↔atroofia) aluseks on skeletilihaskoe morfofunktsionaalsed ja ainevahetuslikud eripärad, mis annavad skeletilihasele võimaluse suurtes piirides adapteeruda rakendatud koormusele või selle puudmisele.

Kuna skeletilihase nii atroofia kui ka hüpertroofia uuringud inimestel on komplitseeritud mitmetel põhjustel, kasutatakse seetõttu mitmesuguseid lihasatroofia/hüpertroofia mudeluuringuid katseloomadel.

Atroofia esilekutsumiseks ja mehhanismi uurimiseks skeletilihases kasutatakse suspensiooni, antigravitatsiooni, denervatsiooni, immobilisatsiooni ning erinevaid hormoonpreparaate. Kompensatoorse hüpertroofia esilekutsumiseks ning uurimiseks kasutatakse sünergistlihaste tenotoomiat, ablatsiooni (sünergistlihase osaline või täielik eemaldamine), passiivset lihase venitust ning erinevaid treeningumudeleid.

Üheks oluliseks ravimite rühmaks, mis võeti kasutusele juba eelmise sajandi viiekümnendatel aastatel, on glükokortikoidid. Farmakoloogilisest aspektist on kõige tähtsamad glükokortikoidide põletiku- ja allergiavastane toime, mida kasutatakse edukalt mitmete haigusseisundite ravis, sealhulgas omavad nad palju kõrvaltoimeid, nad võivad põhjustada Cushing'i sündroomi, muutusi rasva-, valgu- ja süsivesikute ainevahetuses ning sellega võib kaasneda lihasnõrkus ja – kõhetumine ehk lihasatroofia.

Käesoleva töö eesmärgiks oli uurida lihasatroofia arengut glükokortikoidse hormooni manustamisel lihasraku histoloogilistele parameetritele ja kontraktiilsete valkude ekspressioonile ning funktsionaalse ülekoormuse mõju atrofeerunud skeletilihase struktuurile ja koevalkude ainevahetusele.

Töö tulemused näitasid, et glükokortikoidide manustamine kutsub esile kiiret tüüpi lihasrakkude atroofia, mis väljendus erinevate morfoloogiliste ja histoloogiliste parameetrite muutuses (nurgelised lihasrakud, vähenenud lihasraku ristlõikepindala, lihasrakkude lõhenemine, sidekoe vohamine lihasrakkude vahel).

Funktsionaalne ülekoormus kutsus atrofeerunud lihaskoes esile muutusi müosiini raske ahela isovormilises kompositsioonis, kus kiireid isovorme ekspresseeriti ka aeglases lihases (SOL) ning intensiivsemalt kahte kiiret isovormi (MyHC IIb, MyHC IId) kiires lihases (PLA). Samal ajal ei vähendanud funktsionaalse ülekoormuse rakendamine lihasatroofia tingimustes olulisel määral kahjustunud koestruktuuride patoloogilisi karakteristikuid.