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General Review

Turnover of Skeletal Muscle Contractile Proteins in Glucocorticoid Myopathy

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Muscle weakness in glucocorticoid myopathy results mainly from muscle atrophy, the reason for which is the accelerated catabolism of muscle proteins. As the content of lysosomes in skeletal muscle, particularly in fast-twitch glycolytic fibers, is relatively low the non-lysosomal pathway makes a particularly significant contribution and has special importance in the initial rate-limiting steps in the catabolism of contractile proteins and in the regulation of their turnover rate. The turnover rate of actin and the myosin heavy chain is decreased in all types of muscle fibers, and more rapid turnover of the myosin light chain is registered in the fast-twitch glycolytic and oxidative-glycolytic fibers. Exercise and simultaneous glucocorticoid treatment is an effective measure in retarding skeletal muscle atrophy and provides protection against muscle wasting.

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Administration of large doses of glucocorticoids induces muscle myopathy [1, 2]. The fluorinated glucocorticoids appear to produce more extensive muscle atrophy and clinical myopathy than do other steroid types [3, 4].

A number of mechanisms have been proposed to explain the action of chronic glucocorticoids on skeletal muscle. As glucocorticoid treatment alters muscle protein and carbohydrate [5] metabolism, in most studies it was suggested that the main reason for muscle weakness and wasting may result from altered muscle metabolism with net protein catabolism [6, 7]. It was also shown that glucocorticoids might reduce mitochondrial respiration [5, 8] and the excitability of skeletal muscle sarcolemma [9, 10], the suggestion being that the difference in response to steroid treatment may reflect structural and functional differences in fast and slow muscle membrane systems [11]. Later it was demonstrated that hormone treatment did not produce muscle weakness by impairing sarcolemma excitability or excitation—contraction coupling, but the weakness resulted from muscle atrophy [12]. As a decrease in the *in vitro* resting membrane potential of muscle fibers was recorded from only the extensor digitorum longus muscle but not in other muscles composed predominantly of fast-twitch fibers, and as glucocorticoid treatment did not produce any alteration in the *in vivo* measured resting potentials or excitability of extensor digitorum longus muscle fibers, it did not appear that this depolarization was functionally significant [12].

It is well known that Cushing's syndrome leads to a marked reduction in muscle mass, wasting of muscle and loss of strength. Iatrogenic steroid myopathy as well as Cushing's disease suggest a selective atrophy of fast-twitch muscle fibers [3]. It was clearly demonstrated that corticosteroid myopathy is accompanied by changes in the turnover rate of muscle proteins [1, 6, 13]. Further investigations, however, have shown that major changes take place in myofibrillar protein turnover [14–17].

One of the most unclear spheres in protein metabolism is protein turnover, particularly its functional significance. Protein turnover, which is controlled by rates of protein synthesis and degradation, is the integral index of protein metabolism. A crucial problem in the study of protein metabolism in skeletal muscle is the physiological significance of different turnover rates for functionally related myofibrillar proteins. There are various opinions about the relative turnover rate of myofibrillar proteins. In some studies mutual differences in the turnover rate of muscle

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contractile proteins were shown [18, 19]. In others it has been suggested that the differences found in the turnover rate of myofibrillar proteins are due to methodological errors [20]. In our opinion the main confusing point in this problem is the fact that different procedures have been used to separate individual myofibrillar proteins. Therefore, to eliminate methodological doubts, myofibrillar proteins should be separated by a single step procedure. As has been shown [18], this method allows the determination of the relative turnover rate of these functionally related proteins in muscle.

Another complicated question is tracer administration. There are not many studies but they are convincing and show the double-isotope method to be more suitable for the determination of the turnover rate of myofibrillar proteins [19, 21]. Double-isotope methods permit comparison of the turnover of several proteins having the same intracellular origin. As has been shown, tracer administration must be chosen so that the cells or tissues are exposed to the first isotope over the time necessary for the specific radioactivity of a protein to pass the crossover point. It should reflect the descending limb of the specific radioactivity of a protein [21]. The second isotope should be administered just before the experiment is terminated so that it will reflect the ascending limb of the specific radioactivity of a protein [21]. Therefore, the higher the radioactivity of the protein during the labeling period prior to the crossover point, the higher will be its turnover [21].

Expressing the incorporation data as a ratio of the activity of the two isotopes is a more sensitive index of relative protein turnover than comparing the two levels of labeling with only one isotope. The main advantage of the double-isotope method seems to be its simplicity and reliability. It is based on the necessity to compare the determination of the ratio of the two isotopes in a single sample of purified myofibrillar protein. To date information about changes in the turnover rate of myofibrillar proteins in different types of muscle fibres in corticosteroid myopathy has been gained from animal experiments only. The same information about humans has remained at the level of whole muscle.

Glucocorticoids decrease the *in vivo* rate of incorporation of amino acid into the myosin heavy chains, and they likewise decrease the turnover rate of the myosin heavy chains [16]. In dexamethasone-treated rats the myosin light chain synthesis was not affected by glucocorticoids, and the light chains turned over more rapidly than they did in the control rats in all types of muscle fibers except in the slow oxidative ones [22]. In the slow oxidative fibers the synthesis of the myosin light chains was inhibited and there were no significant changes in their turnover rate. Dexamethasone administration decreased the relative weight of the fast-twitch glycolytic fibers [23]. It is possible that the slow-twitch muscle fibers are less sensitive to the action of glucocorticoids, but on the molecular level myosin heavy and light chains seem to have different sensitivity to the hormones.

The synthesis of actin was inhibited in all types of muscle fibers in dexamethasone-treated animals and its turnover rate was slower than in the control animals [22]. The relative turnover rate of actin is significantly decreased in all types of muscle fibers during dexamethasone treatment. The turnover rate of myosin heavy chains was lower in the fast-twitch muscle fibers. A comparison of the myosin light chains turnover rate with the turnover rate of the myosin heavy chains and actin shows that the myosin light chains have a more rapid turnover rate in all types of muscle fibers [16].

It is not surprising that the slow- and fast-twitch skeletal muscles differ in their response to the hormone, but it is surprising that in the fast-twitch fibers large doses of glucocorticoids cause a more rapid turnover rate of myosin light chains. On the other hand, the myosin heavy chains turnover was more significantly decreased in the fast-twitch muscle fibers than in the slow-twitch ones [16]. Glucocorticoids do not diminish the intensity of protein synthesis in heart muscle and slow oxidative skeletal muscle fibers, but do so in the fast muscle fibers, especially in the fast glycolytic ones [16, 24, 25]. The actual reason for this is so far unknown.

Glucocorticoid excess has been shown to depress plasma testosterone levels [26] and may alter circulating concentrations of other hormones such as insulin [14]. The lack of testosterone causes a decreased capacity for protein synthesis similar to that observed with glucocorticoid treatment [27]. As contractile proteins in different types of muscle fibers have different sensitivity to hormones [28], changes in the hormonal milieu may be one of the reasons for changes in protein synthesis in muscle fibers of different metabolic types in corticosteroid myopathy. During glucocorticoid treatment a reduced incorporation rate of amino acids into myofibrillar and sarcoplasmic proteins [29] or only into myofibrillar proteins [16] was observed in skeletal muscle. The muscle contains polysomes of different sizes which code for the synthesis of various myofibrillar proteins [20, 30]. The myosin light and heavy chains, which together constitute myosin, are translated independently by different monocistronic mRNA. The heavy chains of myosin are synthesized on polyribosomes containing 60–80 ribosomes by a 26S mRNA; the light chains are coded for by another mRNA from polyribosomes containing 4–8 ribosomes [31, 32]. Following the synthesis of different sub-units of myosin on the separate mRNA, the polypeptides are assembled to form the native myosin molecule [32]. Glucocorticoids may also reduce skeletal muscle protein synthesis in part by regulating the activity of factors involved in peptide-chain initiation, and that factor activity seems to be linked to tissue RNA content [33]. Glucocorticoid treatment

leads to a decrease in polyribosomal and total RNA in rat muscles [17, 25]. Ribosomes isolated from the hindlimb muscle of rats treated previously with triamcinolone acetonide have a reduced ability to incorporate amino acids into protein *in vitro* [26, 34].

The main reason for the development of muscle atrophy in corticosteroid myopathy is the accelerated catabolism of muscle proteins [6, 14, 35]. It is well established that lysosomal and non-lysosomal pathways also exist in skeletal muscle to account for the degradation of their intracellular proteins. As the content of lysosomes in skeletal muscle is relatively low, the non-lysosomal pathway makes a particularly significant contribution and may be of special importance in the initial rate-limiting steps in the catabolism of myofibrillar proteins and consequently in the regulation of their turnover rate. A promoted breakdown of contractile proteins myosin and actin in the muscle during glucocorticoid administration was shown by the enhanced excretion of 3-methylhistidine [13]. Several studies have demonstrated that the slow-twitch skeletal muscles are much more resistant than the fast-twitch muscles to any catabolic action of the corticosteroids [6, 23, 25]. The catabolic effect of glucocorticoids on myofibrils seems to be realised through the augmented alkaline myofibrillar activity [22]. On the other hand, it is possible that the myosin heavy chains and actin are more sensitive to the action of alkaline myofibrillar protease than are the myosin light chains, at least under *in vitro* conditions [17]. As has been pointed out, the weight reduction in different muscle types in dexamethasone-treated animals is in full accordance with the augmentation of their alkaline protease activity [23, 36]. Although a significant weight reduction occurred only in the fast-glycolytic fibers, the intracellular catabolic effect of glucocorticoids was noted in the fast oxidative-glycolytic and slow oxidative muscle fibers as well.

Alkaline proteases are synthesized in mast cells. After degranulation the enzyme enters the muscle cell, but the mechanism is unknown. Upon administration of large doses of glucocorticoids there is an increase in the number of mast cells in the perivascular porous connective tissue of the muscle fibers [37]. Around the fast glycolytic muscle fibers, degranulation of mast cells is very clearly expressed [37]. Simultaneously the number of mast cells in the lymph node medulla is considerably decreased. The lymph nodes are probably the sources of the muscle mast cells. This may imply that the increased number of mast cells may be the result of their migration from the lymph nodes. Forty-eight hours after glucocorticoid administration the mast cell number in lymph nodes is returned to control levels [37]. In the atrophying muscle, myofibrillar destruction starts from those myosin filaments which are located in the peripheral part of the myofibrills [35, 38]. Thick filaments separate from the adjacent ones, bend and are obviously lysed [35]. The actin filaments and Z-line seem to be more resistant to the action of alkaline protease, in comparison with the myosin filament [35, 38]. Muscle weakness in the case of glucocorticoid myopathy is most probably caused by lesions of the myofibrillar apparatus in the muscle fibers and by changes in the state of the neuromuscular synapses [39].

The catabolic action of glucocorticoids on skeletal muscle was found to depend on the functional activity of skeletal muscles [6, 23]. Several studies have demonstrated that endurance exercise with simultaneous glucocorticoid treatment is an effective measure in retarding skeletal muscle atrophy [40–42] and provides protection against one of the major effects of glucocorticoids, i.e. muscle wasting [43]. Endurance exercise with simultaneous glucocorticoid treatment in rats causes a decrease of muscle mass, but in comparison with unexercising animals the mass of extensor digitorum longus and gastrocnemius muscles was subsequently 57 and 48% higher [15].

It was shown that in exercising glucocorticoid-treated rats the synthesis rate of actin in fast oxidative-glycolytic muscle was 65% and in fast glycolytic fibers 49% more intensive than in hormone-treated animals. At the same time synthesis of myosin heavy chains in fast glycolytic fibers was 14% more intensive [15]. It seems that exercise causes an anabolic effect in both types of fast-twitch muscle fibers mainly by intensification of actin synthesis rate. In exercising glucocorticoid-treated animals significant intensification in the synthesis rate of myosin heavy chain was only observed in fast glycolytic fibers. Glucocorticoids can inhibit or diminish the action of androgens, and androgenic action may partly be involved in limiting or inhibiting glucocorticoid effects [40].

The effect of physical activity in inducing a less pronounced catabolic action of corticosteroids seems to be caused by the elevation of anticatabolic activity of exercise [15, 23]. Also, the role of endogenous androgens cannot be excluded from the anticatabolic effect of the exercise, since only moderate exercise had an anticatabolic activity in skeletal muscles of corticosteroid myopathic rats. Exhaustive exercise, on the contrary, augmented the catabolism in skeletal muscles [23]. At the same time, anabolic steroid in these situations elevated the anticatabolic activity in the fast-twitch glycolytic muscle fibers [17, 22]. Fast-twitch glycolytic fibers are as sensitive to the catabolic action of glucocorticoids as to the anabolic action of anabolic steroids [17, 22].

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