[Frontiers in Bioscience E4, 230-239, January 1, 2012]

The effect of exercise on IGF-I on muscle fibers and satellite cells

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1. ABSTRACT

Skeletal muscles are able to adapt to over - or under-use. In response to enhanced use, such as exercise, skeletal muscles undergo morphological and physiological changes possibly including injury and regeneration of muscle fibers (myofibers). The regeneration process includes addition or replacement of myofiber nuclei (myonuclei) (1). Myonuclei are terminally differentiated, thus maintenance and repair of myofibers are attributed to satellite cells, the myogenic stem cells. Up to date little is known about the differential effects of different growth factors on satellite cell and their subsequent contribution in exercise. The adaptation of skeletal muscles to altered use is governed by three major processes: satellite (stem) cell activity, gene transcription, and protein translation. A defect in any of these processes could interfere with muscle maintenance and regeneration. This review focuses on current understanding of the effects of resistance and endurance exercise on skeletal muscle fibers (myofibers) and on the skeletal muscle stem cells, satellite cells. We first summarize in brief the basic biology of skeletal satellite cells; the types of exercise and the basic biology of IGF-I. We then discuss the interplay between IGF skeletal muscle and satellite cells.

2. MYOFIBERS AND RESIDENT STEM CELLS, SATELLITE CELLS

Myofibers are the functional contractile units of skeletal muscles. A myofiber is a multinucleated single muscle cell (Figure 1) that about 85% of its volume is composed of contractile proteins such as actin, myosin and titin (the latter is the largest protein in the muscle, responsible for muscle elasticity) (2, 3). Each myonucleus regulates gene transcription and protein synthesis within a certain volume of cytoplasm termed "myonuclear domain" (4, 5). Events, such as oxidative stress, that alter the balance between protein synthesis and degradation contribute to increasing or decreasing the volume of myofibers (i.e., hypertrophy or atrophy, respectively). It was shown that in response to exercise, gene transcription is activated within seconds from the contraction initiation and can last hours after the cessation of exercise (6). Nuclei of adult myofibers are post-mitotic, thus growth and regeneration of skeletal muscles depend on a population of cells, termed satellite cells. Satellite cells give rise to myogenic progeny and are able to self renew and are thus considered the skeletal muscle stem cells (7); it is not established yet, however, if all satellite cells possess self-renewal capacity (8). Satellite cells are typically quiescent,

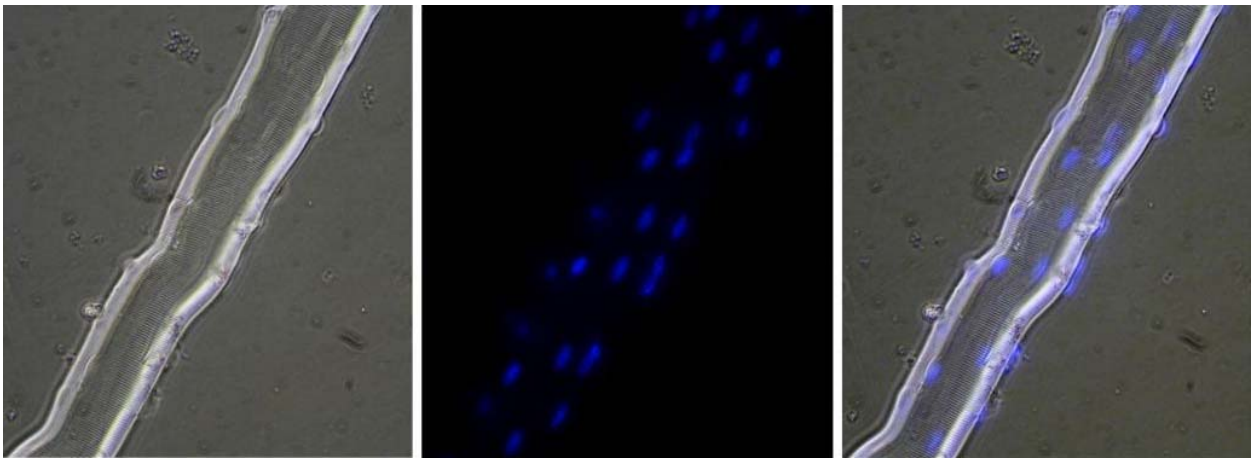

Figure 1. Myonuclei along the length of an isolated muscle fiber. A. contrast image of a myofiber. B. Nuclei are stained with DAPI (a florescent dye that binds the DNA). C. A merged image of the myofiber and its myonuclei.

neither differentiate nor undergo cell division, but are activated and recruited to proliferate and participate in regenerative response upon subtle injuries such as exercise and work overload, or as a result of major trauma (1, 9-11). Functional satellite cells that give rise to myogenic progeny are present even in myofibers of senile and old animals and humans. However, the numbers of satellite cells are lower in myofibers from old and senile compared to those from young animals and humans (12-14).

Satellite cells are distinguished from myonuclei or other cells in the muscle tissue based on their unique expression of the paired-box transcription factor Pax7 (15-17). Upon their activation, satellite cells enter the cell cycle and contribute new myogenic progeny (that either add more myonuclei or fuse with each other to form new myofibers). The process of satellite cell proliferation and differentiation is regulated by a family of muscle specific transcription factors (also known as myogenic regulatory factors or MRFs) that includes Myf5, MyoD, myogenin, and MRF4 (8, 18, 19).

3. TWO TYPES OF EXERCISE

Exercise can be grossly divided to two types that differ in (a) the duration and intensity of muscular contractions and (b) the dependence of muscles on oxygen for generating the energy needed for contraction.

Aerobic/ endurance exercise involves long periods of activity in which muscles contract below their maximal ability (for example, marathon run). In this type of physical activity muscles consume large amounts of oxygen to generate energy primarily from breaking down carbohydrates. In the absence of carbohydrates muscles switch to fat metabolism. The latter is accompanied with a decline in muscle performance.

Anaerobic/ resistance exercise refers to short bursts of high intensity muscle contractions. For energy, glycogen or glucose are metabolized with relatively little oxygen. This anaerobic glycolysis involves the formation of by-products such as lactic acid that are disadvantageous to muscle function. In a second form of anaerobic exercise, which lasts less than a minute, creatine phosphate is used as the main source for muscle energy. Typically, anaerobic exercise induces marked muscle hypertrophy by inducing synthesis of structural proteins. Endurance training induces enhanced glucose metabolism and mitochondrial activity; some evidence point to protein synthesis but to a lesser extent compared to resistance training (20, 21).

4. INSULIN GROWTH FACTOR (IGF-I) STRUCTURE AND FUNCTION

Insulin growth factor 1 (IGF-I) also known as somatomedin C, is a hormone that plays fundamental roles both in prenatal development and in postnatal life. IFG-I affects many metabolic and anabolic processes, cell growth, differentiation and survival (22-24). IGF-I shares structural homology and downstream signaling pathways with insulin and can facilitate glucose and free fatty acid uptake, enhance protein synthesis and suppress protein degradation (25-27). IGF-I protein is composed of three α-helices made of 70 amino acids with three intra chain disulfide bonds. IGF-I binds to the IGF-1 or the insulin cell surface receptors (IGF1R or IR, respectively).

The circulating form of IGF-I is produced by the liver in response to the binding of the growth hormone (GH) to its hepatic receptor (28). IGF-I has high affinity for IGF-I binding proteins (IGFBPs) (29), only about 2% of IGF-I circulates as a free form. Most IGF-I circulates in either a binary (~20–25%) or ternary (~75%) complex with IGFBPs. There are six IGFBPs, of which IGFBP-3 is the most abundant and accounts for 80% of bound IGF-I. The autocrine/paracrine form of IGF-I, which is not produced by the liver, has relatively low affinity to IGFBPs (29-31).

The expression of the IGF-1 gene is activated in response to many signals, for example testosterone and growth hormone and inhibited by glucocorticoids and proinflammatory cytokines such as TNFα and interleukin-

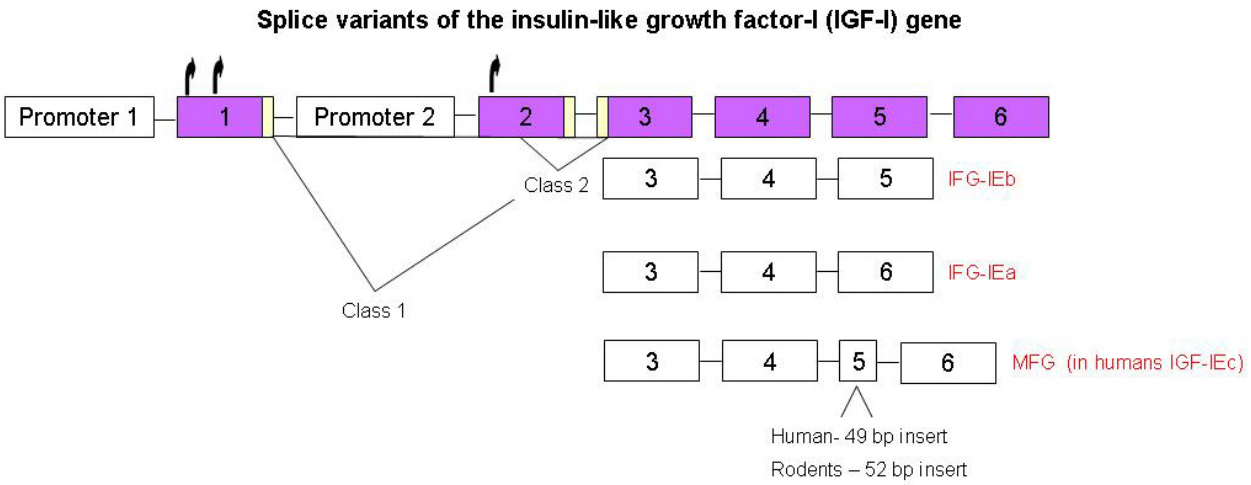

Figure 2. Schematic representation of rodent IGF-1 gene. The gene is composed of six exons (colored boxes), separated by introns (black lines). Multiple transcription start sites (Ì) on exons 1 and 2. Exons 1, 2 and 3 code for the signal peptide of precursor IGF-I. Exons 5 and 6 each encode either IGF-IEb or IGF-IEb, respectively.

1. In mammals, the IGF-I gene is composed of at least 6 exons, has several transcription start sites positioned on exons 1 and 2 and undergoes alternative splicing resulting in several variants that are translated to smaller E peptides (32, 33) (Figure 2). Transcripts that contain exon 1 or exon 2 are termed IGF-I class 1 or 2, respectively. Exon 4 encodes the first 16 amino acids shared by all E peptides, the rest of the amino acids are encoded by exon 6 (Ea), or exon 5 (Eb), or an insert composed of the first, 49 base pairs of exon 5 in humans or first 52 base pairs in rodents, spliced onto exon 6 (MGF). The first discovered isoform was IGF-IEa, it is often referred to as liver-type or systemic IGF-I albeit it is produced in other tissues as well (34, 35). This isoform plays an important role in the process of muscle hypertrophy that occurs in response to overload and resistance exercise. IGF-IEb in rodents and IGF-IEc in humans are produced mainly by skeletal muscles, and are often referred to as mechano growth factor (MGF) because of the enhanced expression in response to mechanical stimuli such as stretch (36-39).

5. IGF-I AND MUSCLE

IGF-I operates throughout prenatal and postnatal periods. Mice lacking the IGF-I receptor exhibit marked muscle hypoplasia (muscles composed of below-normal number of cells) and die soon after birth because they failed to breathe (40). On the other hand, mice over-expressing IGF-I exclusively in their muscles develop muscle hypertrophy (41-43). At the skeletal muscle level, IGF-I was suggested to have multiple beneficial roles. For example, muscle-specific expression of IGF-I was suggested to block age-related loss of muscle function in mice (41) and to mitigate the pathology (44) and improve muscle function (45, 46) in a mouse model of Duchenne muscular dystrophy (dystrophin-deficient mdx mice). The specific effects of IGF-I on myogenic differentiation (myogenesis) were studied extensively over the years. It is believed that IGF-I has a dual role in myogenesis, when inducing myoblast proliferation it activates the extracellular signal-regulated kinases 1/2 (ERK 1/2) pathway and when inducing differentiation it activates the phosphoinositol 3-kinase/Akt signaling path (47, 48). The most impressive effect of IGF-I in skeletal muscles is the induction of hypertrophy that was demonstrated in multiple model systems including transgenic over-expression, viral-mediated delivery, directed infusion, and plasmid injection (41-43, 49). It appears that IGF-I peptide regulates muscle mass by: inducing protein synthesis, inhibiting protein degradation, and by stimulating proliferation and fusion of satellite cells to existing myofibers (50-52). With the most convincing result that over expression of IGF-I is sufficient to induce muscle hypertrophy and the necessary increase in protein synthesis, decrease in protein degradation, and activation and fusion of satellite cells (42, 43). There is a consensus on the essence of IGF-I as a major regulator of muscle mass in prenatal development and during growth and regeneration when growth stimuli support the formation of new myofibers (53). There is debate, however, regarding the importance of IGF-I in regulating the mass of intact muscles in adults. In recent years accumulating evidence support the notion that IGF-I is not the prime regulator of muscle hypertrophy induced by mechanical overload (i.e., typically resistance/strength exercise). For example, hypertrophy occurred even in the absence of (a) circulating IGF-I (when IGF-I was inactivated exclusively in the liver) (54) and (b) functional muscle IGF-IR (due to expression of a muscle-specific dominant negative IGF-IR) (55). Moreover, it was shown that when the signaling of the IGF-I receptor was abolished and that of insulin was drastically reduced there was a 30% reduction in the muscle mass of young (21 day old) mice. However, as these mice matured, the reduction of muscle mass became less apparent (56). Additionally, it was recently shown that forced increase of IGF-I levels enhance hypertrophy in intact growing but not in adult mice (53).

IGF-I has an anabolic effect on skeletal muscle mostly owed to its role in promoting protein synthesis. This

effect is achieved via the activation of Akt/TSC2/mTOR pathway that induce protein synthesis and Akt and Foxo transcription factors that suppress protein degradation by down regulating two E3 ubiquitin ligase genes, atrogin-1 and muscle ring finger-1 (MuRF-1) (57).

Differently, in conditions of physiological or induced muscle waste, for example as a result of hindlimb suspension or paraplegia, the activation of mTOR and Akt are reduced and so is the abundance of total Akt protein (58-61). Apparently these effects are attributed to local rather then systemic IGF-I, since muscle specific infusion or genetic manipulation to increase muscle levels of IGF-I were sufficient to induce hypertrophy (unlike systemic administration of IGF-I and/or GH) (43, 49, 62). In the same vein, the process of hypertrophy was not altered in rats lacking GH due to surgical ablation of their hypophysis (62) and circulating IGF-I levels did not change in humans after a single bout or 10 weeks of resistance exercise (63, 64). It should be noted, though, that mTOR signaling can also be activated by mechanical strain independent of the activation of the IGF-IR or PI3K signaling (55, 65).

6. IGF-I AND EXERCISE

Tight relation between muscle hypertrophy, IGF-I levels and exercise was shown in many studies when inflicted overload exerted dramatic effects on muscle hypertrophy and IGF-I levels. Moreover, it appears that skeletal muscles depend on autocrine and/or paracrine (i.e., local in the muscle tissue) IGF-I rather than liver-derived IGF-I for proper adaptation (and maybe regeneration). This is based on studies showing that (a) overload or resistance training induce increased local, muscle, production of IGF-I in rodents that lack serum IGF-I due to surgical removal of the hypophysis (hypophysectomy) or genetic manipulation; and (b) exogenous administration of GH or IGF-I, which increase serum IGF-I levels, does not stimulate myofiber hypertrophy in the absence of mechanical load (54, 75-77).

Muscle stretch resulted in local upregulation of IGF-I and hypertrophy of rat muscles (66). In humans, mRNA levels of IGF-I increased after one bout of resistance exercise (67) and protein expression was enhanced after 12 weeks of training (68). IGF-I levels in the serum were shown to decline with age but to increase in response to resistance and endurance exercise (38, 69-74). A closer examination of the effect of exercise and age on the different IGF-I splice variants revealed, both in humans and rodents, different dynamics. Intense resistance exercise, as well as muscle damage, resulted in rapid upregulation of MGF (peaking within 24 hours) while IGF-IEa upregulation peaks only after 1-3 days (78-84).

The length of the exercise period determined the effects on the expression levels of IGF-I splice variants in the muscles of young and old subjects. Short term resistance training induced upregulation of MGF mRNA levels in the muscles of young and adults but not in old rodents and humans (80, 84-86). IGF-IEa mRNA levels, however, were not affected by one bout of resistance exercise in young and old subjects (80, 86). Prolonged resistance training (12 weeks), on the other hand, induced upregulation of IGF-IEa and MGF in muscles of old subjects (82-84, 87).

Some studies claim that MGF is more effective in inducing muscle hypertrophy than IGF-IEa because MGF affects the initial activation of satellite cells (88), others decline the preferential effect of MGF on hypertrophy (35). Specifically, Barton and coauthors (35) used a technique of gene-transfer adeno-associated virus (AAV) of MGF and IGF-IEa and did not find differences in the degree of hypertrophy two and four months after virus injection into the extensor digitorum longus (EDL) muscle of very young mice. Hypertrophy was noted, however, only in 6 months old mice that were injected with IGF-IEa AAV.

It is typically accepted that IGF-IEa and MGF probably mediate different responses of muscle cells to external stimuli. For example, it was suggested that MGF expression is preferentially responsive to mechanical stimuli, whereas IGF-IEa is more responsive to metabolic challenges. It was also suggested that the IGF-I gene is spliced to produce the MGF transcript as an immediate response to muscle injury, prior to satellite cell activation. While a few days after muscle injury the IGF-I gene splices more towards the IGF-IEa isoform (79). If indeed MGF is more responsive to mechanical stimuli it is reasonable to assume that resistance exercise will preferentially inflict increase in the MGF isoform while endurance will preferentially inflict increase in IGF-IEa isoform. Up to date no studies addressed the differential expression of the two splice variants in response to resistance versus endurance exercise but rather focused on resistance exercise only. Moreover, to date little is known about the influence of growth factors such as IGF-IEa and the splice variant MGF on bona-fide satellite cells in response to prolonged endurance exercise training.

7. IGF-I AND SATELLITE CELLS

While there is a consensus about the anabolic effects of IGF-I in promoting muscle hypertrophy, the effects of IGF-I on muscle satellite cells may be less evident. These effects were investigated in many studies by examining myogenic immortalized cell lines. Here we mainly concentrate on knowledge accumulating from studies analyzing satellite cells in muscle sections (in-vivo) and studies with primary myogenic cells. This is in light of several differences between cells lines and primary cells which make the deduction from results obtained from experiments with cell lines to bona-fide satellite cells difficult. The most striking difference is that cell lines typically proliferate indefinitely and do not undergo senescence, probably due to different genetic and/or epigenetic regulation, compared to primary cells. The extensively used myogenic cell lines (the mouse C2 and its subclone C2C12 and the rat L6) were established 30-40 years ago (89, 90). Yet there is no characterization of (a) the changes these cells may have underwent since their establishment or of (b) the changes between the same cell

lines used by different laboratories. Moreover, it is known that the biology of myogenic cell lines does not mirror that of satellite cells in terms of expression of master regulatory genes and the response to growth factors, including IGFs (91).

Exercise and injury were shown to enhance the expression and activity of IGF-I, IGF-I receptors and IGF-I-activated signaling pathways. Skeletal muscle regeneration following damage and adaptation to exercise are mainly attributed to activation, proliferation and myogenic differentiation of satellite cells. This is followed by fusion of satellite cells either with each other (forming new myofibers) or fusing with the surviving damaged myofibers (8). During muscle regeneration the expression of IGF-I was detected in the cytoplasm of myoblasts and myotubes as well as in satellite cells (92). Similar to its effects on regeneration, IGF-I was suggested to be involved in the exercise-induced proliferation of satellite cells (10). An earlier study showed increased protein expression of IGF-I in muscle samples from humans that performed a week of intense military training (93). In this study, IGF-I was found in cells that may be satellite cells. A link between IGF-I, satellite cell numbers and hypertrophy was indicated in a study where IGF-I was administered locally to the tibialis anterior (TA) muscle of adult rats. This resulted in increased total DNA and protein content, suggesting an increase not only in protein but also in the content of nuclei that are contributed by satellite cells (49). In addition to satellite cell proliferation, IGF-I was shown to stimulate differentiation and to promote subsequent fusion with existing myofibers (94). Supporting the view that hypertrophy involves the effect of IGF-I on satellite cell myogenesis is a study showing that IGF-I induced only modest hypertrophy when satellite cells were ablated from muscles upon γ-irradiation (95). There is evidence that the process of muscle hypertrophy encompasses the effect of IGF-I not only on satellite cells but also on their parent myofibers. Specifically, it was shown that IGF-I binds to receptors situated on myofibers as well as on activated single primary myogenic cells (56, 96, 97). Lately, attention was paid to the expression and action of the different splice forms of IGF-I in primary myogenic cells (including satellite cells) and myofibers. It was shown that 16 weeks of resistance exercise induced upregulation of MGF, myofiber hypertrophy, increase in satellite cell number and myonuclear addition (98, 99). If and what IGF-I splice variant is involved in the activation of satellite cells or affect already activated satellite cells is not yet resolved. Some studies with rodents and humans show that, upon muscle challenge, there is preferential splicing of the IGF-I gene towards MGF which coincides with muscle stem cell activation (79, 80, 82, 100). The involvement of MGF in satellite cell activation (i.e., not in myogenic cell lines) was based on two studies (79) in which the tibialis anterior (TA) muscles of rats were damaged by physical or chemical means and the regeneration of these muscles was analyzed up to 24 days post injury. Results showed that: (a) mRNA expression levels of MGF were upregulated prior to the levels of IGF-IEa; (b) levels of MGF correlated with the upregulation of M-cadherin, a marker of satellite cells, and MyoD which is a muscle specific transcription factor expressed in activated satellite cells; and (c) the levels of MGF peaked before M-cadherin (this was verified also at the protein level). IGF-IEa levels, however, peaked only after M-cadherin levels reached a plateau. Based on these results authors suggested that the product of the MGF splice variant accounts for the activation of satellite cells.

Supporting the notion that MGF plays a role in satellite cell activation is another study showing that addition of synthetic MGF peptide enhanced the proliferation of cultured human primary muscle cells obtained from healthy subjects as well as from muscular dystrophy and ALS patients (101). The main pitfall of this study is that activated satellite cells were identified based on desmin expression - however this protein is expressed (a) both in quiescent and proliferating satellite cells (102, 103); and (b) not specifically in skeletal muscle cells (104, 105). Thus the direct role of this synthetic IGF splice variant on the activation of bona-fide satellite cells remains open to doubt. Another set of studies suggest that MGF affects satellite cells that are already activated. Specifically, a study with 2-week and 6-months-old mice in which the TA and (EDL) muscles were injected with recombinant adenovirus containing a vector encoding the murine IGF-IEb (MGF) showed that four months after injection significant muscle hypertrophy was evident in the 2 week but not 6-months old mice. Based on these results authors postulated that when active satellite cells are diminished, such as in the 6-month-old animals, IGF-IB cannot promote hypertrophy (35). This study, unlike the previous ones does not analyze challenged muscles that typically encompass activated satellite cells. This is because the recombinant virus DNA is single stranded and requires complementation before expression which delays the expression of the vector about 2 weeks post-injection. This means that by the time the vector (i.e., IGF-IB) is expressed muscle repair is well underway, and the pool of activated satellite cells is reduced. The temporal separation between damage due to injection and expression of IGF-IB limits the population of activated satellite cells to those involved in normal maintenance (35).

In an attempt to investigate the effect of the MGF peptide, rather than transcript, several studies used a synthetic Ec peptide, described in (106). It is very important to note that the peptide used by Yang et al (106) does not correspond to the natural Ec peptide but is a shorter peptide encoded by exon 6. This “short” Ec peptide was shown to induce proliferation and inhibit differentiation of the myogenic cell line C2C12 myoblasts (106) and human myoblasts (101, 107), and to result in increased in motility of myoblasts injected into muscles (108). Taken together these results suggest that the Ec peptide stimulates proliferation and inhibits differentiation of myogenic cells, as does expression of the full-length MGF construct (106) in muscle cells.

In summary, despite a wealth of studies, most of the current knowledge about the effect of the different splice forms of IGF-I on satellite cells in response to exercise or injury is based on correlations between IGF-I expression and markers of satellite cell activation. Until

direct evidence that link between IGF-I and bona-fide satellite cell function our knowledge remains limited.

8. ACKNOWLEDGMENT

This review was supported by a grant from the EU-FP7, Excell project 214706.

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Key Words: satellite cells, skeletal muscle, exercise, myofibers

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