Age-associated Changes in the Response of Skeletal Muscle Cells to Exercise and Regeneration

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ABSTRACT: This paper looks at the effects of aging on the response of skeletal muscle to exercise from the perspective of the behavior of muscle precursor cells (widely termed satellite cells or myoblasts) and regeneration. The paper starts by outlining the ways in which skeletal muscle can respond to damage resulting from exercise or other trauma. The age-related changes within skeletal muscle tissue and the host environment that may affect the proliferation and fusion of myoblasts in response to injury in old animals are explored. Finally, in vivo and in vitro data concerning the wide range of signaling molecules that stimulate satellite cells and other aspects of regeneration are discussed with respect to aging. Emphasis is placed on the important role of the host environment, inflammatory cells, growth factors and their receptors (particularly for FGF-2), and the extracellular matrix.

EXERCISE AND REGENERATION

There are at least three possible cellular responses that can occur in muscles subjected to exercise as outlined below.

Low-level “Sublethal” Damage Insufficient to Provoke Regeneration

Specific forms of exercise, in particular lengthening contractions (eccentric muscle actions, e.g., those that occur when descending any incline), can result in disruption of the myofibrillar structure, especially that of the Z-bands, and also in minor membrane damage, resulting in “leakiness” of the sarcolemma. It seems that such minor or sublethal injury to myofibers can be repaired locally by rapid restoration of the wounded sarcolemmal membrane so that cellular breakdown is limited and focal necrosis does not occur. The extent to which such sublethal damage occurs after exercise is unknown.

Minor damage does occur in response to modifications in muscle loading causing alterations to myofibrils and nascent sarcomere formation in myotendinous regions associated with an accumulation of macrophages. The macrophages may be specifically attracted to factors and then secrete other factors that assist in remodeling in this area, and yet this inflammatory activity at the myotendinous junction occurs without any associated myofiber necrosis and regeneration. In such situations of sublethal damage there should be no need for satellite cells (myoblasts) to undergo replication. However, Darr and Shultz suggested...
suggest that satellite cells may become activated and replicate even on fibers where overt necrosis is not detectable at the light microscopic level, although it is not known whether these proliferating myoblasts actually fuse under conditions of minor damage (see also discussion below under Hyperlasia versus Hypertrophy). It is worth noting that, in denervated muscle, although there is an increase in satellite cell proliferation above the basal rate, these labeled myoblasts do not fuse but instead subsequently “disappear” from the muscle, either by emigration or cell death. 

**Necrosis and Regeneration**

Where muscle damage is more severe, this will precipitate an influx of calcium ions that results in focal necrosis of myofibers. Such necrosis undoubtedly occurs after intense or unaccustomed physical exercise. In this situation, the damaged area of the myofiber is rapidly sealed off from the remainder of the myofiber by new sarcolemmal formation, observed ultrastructurally at 12 hours after injury and demonstrated at 8 hours by the exclusion of horseradish peroxidase. There is an associated rapid influx of inflammatory cells, followed by satellite cell proliferation and fusion to repair the damaged segment of the myofiber. This is a classical regeneration response. In a quantitative study of muscle injury after exercise, a low level of regeneration was also reported in normal unexercised control adult rat muscle. Although there is good evidence that old muscle is more susceptible than young and adult muscle to injury after exercise, this is not addressed in the present paper.

**Hyperplasia versus Hypertrophy**

The extent to which particular kinds of exercise result in hyperplasia (an increase in the number of muscle nuclei due to satellite cell proliferation and fusion) or in hypertrophy (the classical increase in muscle fiber size due to new protein synthesis) is widely debated. Hyperplasia could result from the fusion of satellite cells with stretched, hypertrophying myofibers, similar to the situation seen with growing muscles during development, in order to maintain some optimal nuclear/cytoplasmic ratio as the fiber increases in size. Although there is very strong evidence of satellite cell proliferation in rat muscle hypertrophying in response to overloading, fusion with the parent myofiber is not always confirmed, and there is also evidence that the satellite cells can form new myofibers (see below). The extent to which such satellite cell proliferation and fusion with hypertrophying parent myofibers does occur within exercised adult muscles is unclear. Much data support the idea that exercise training results in injury that is sufficient to provoke a regenerative response.

Hyperplasia traditionally arises from regeneration (in response to necrosis as outlined above) where satellite cells proliferate and fuse to repair segments of damaged myofibers; in some instances this can result in split or branched myofibers that have the appearance of new myofibers although they are actually continuous with a parent myofiber. There is evidence that genuine new myofibers may be formed *de novo* in interstitial connective tissue between the existing myofibers, from experiments with chicken muscle (hypertrophying in response to weights attached to a wing) and young rat muscle (hypertrophying...
in response to ablation of synergistic muscles). However, the discontinuity of such nascent myofibers can only be proved by serially sectioning the tissue. It is not known if such new myofiber formation occurs in response to exercise. These three situations of hyperplasia (fusion with hypertrophying myofibers, muscle regeneration, and new myofiber formation) require the activation of quiescent satellite cells and their resultant proliferation and fusion. What are the signals that stimulate these events? Are different signals required for these three different situations? Is the availability of the signals or the response of the myogenic cells to these signals affected by age? Before attempting to address some of these questions, the age-related changes within skeletal muscle and the host environment will be examined, because these factors may well influence the behavior of the muscle cells.

**CHANGES WITH AGE**

*Age-related Changes within Skeletal Muscle Tissue*

**Innervation**

Old age is associated with a progressive loss of muscle mass due to atrophy of individual myofibers, as a result of denervation combined with a reduction in the number of myofibers. Old age is also associated with a decrease in force and power due to a loss and change in contractile properties of the motor units in muscle. Because the early events of regeneration (up until fusion) are unaffected by innervation, these age-related changes should have little impact on the capacity for muscle repair after exercise-induced injury. However, other age-related changes within skeletal muscle will have an impact on the regenerative response.

**Extracellular matrix**

Extracellular matrix (ECM) in skeletal muscle includes both interstitial connective tissue and the external (basal) lamina, which is in intimate contact with satellite cells and myofibers. A general increase in interstitial fibrous connective tissue is associated with aging: the amount of endomysial collagen doubles between 3 and 26 weeks of age in mice, and it is well documented that increasing fibrosis occurs in regenerating muscles of older animals. An increase in fibrous connective tissue and “rigidity” will also affect the response of the muscle to exercise. An age-related increase in fibrosis and fibroblastic activity may account for the increased myofiber branching seen in regenerating muscles of old rats. There might also be associated age-related changes in fibroblast-derived soluble growth factors that have been shown to play a paracrine role in myoblast proliferation. Apart from the interstitial connective tissue, an increase in the external lamina encircling satellite cells has been reported with age. In Duchenne’s muscular dystrophy and the animal dystrophies there is a marked increase in extracellular collagen and altered forms of collagen with time (see also ref. 38). Age-related changes in the amount and composition of the ECM components, particularly of the external lamina,
could adversely affect the efficiency of muscle regeneration as discussed later under THE SIGNALS.

Vasculature

Other age-related changes within skeletal muscle relate to the vasculature, with a reduced blood supply, decreased capillary density, and changes in vascular pathology being reported in older subjects. Vascularity and revascularization are affected by many factors, including exercise, and exercise is usually decreased in older subjects. Rapid revascularization can be a major factor in efficient muscle repair, particularly after large injuries, and a decrease in vascularity could have an adverse effect on muscle regeneration, as it could reduce the efficiency of inflammatory cell infiltration, the importance of which is discussed later.

Age-related Changes in the Systemic Host Environment

In addition to such local changes within the muscle tissue itself, there are systemic changes in the complex endocrine system with age. Reduced serum levels of growth hormone and insulin-like growth factor-I (IGF-I) in old humans and rats may have a direct effect on the proliferation and fusion of satellite cells. Other changes in blood-borne factors influence the immune system, and this is of critical importance due to the central role of inflammatory cells during muscle regeneration. Although it was reported that macrophage function and hence muscle regeneration is severely impaired in old compared with young SJL/J mice, this dramatic effect of host age on macrophage function does not occur in other strains of mice and is ablated by orchidectomy. Thus age-related changes in hormonal status can impact on the efficiency of the inflammatory cell response of the host. Delayed macrophage infiltration and associated regeneration after muscle injury was also reported in old (24 month) relative to young rats. Studies in humans show that macrophage activity declines and the extent of mobilization of polymorphonuclear leukocytes (PML) is decreased in older subjects; it is also widely recognized that there is an age-associated decline in T-cell mediated immune parameters and that this can also be affected by certain types of exercise. These influences of the host environment would seem to be of considerable importance in determining the efficiency of muscle regeneration in old animals as discussed below.

Age-related Decrease in Muscle Regeneration

There is clear evidence that muscle can regenerate well in old hosts. However, muscle regeneration in old hosts is generally less successful than in young hosts with respect to both morphological and functional properties (reviewed by Carlson). Although these differences can be subtle in certain situations, although a similar capacity for myoblast proliferation was demonstrated in autoradiographic studies in muscles regenerating after crush injury in old (40 week) compared with young (4 week) host mice, myoblast
replication was retarded in the old hosts. Classical cross-transplantation studies of whole extensor digitorum longus muscles between old (24 month) and young (4 month) rats, and examined at 60 days, showed that it was the age of the host (rather than the muscle graft) that determined the success of regeneration in muscles examined at 60 days. The age-related success in these long-term grafts was attributed to the capacity for axonal regeneration and hence functional reinnervation of the graft. However, experiments in our laboratory suggest that the status of inflammatory cells is another crucial variable in the host environment that influences the success of muscle regeneration. When whole muscle grafts were cross-transplanted between two strains of mice with strikingly different regenerative capacity (SJL/J have superior regeneration compared with BALB/c mice) the pattern of regeneration reflected the strain of the host, again showing that the host environment (rather than the muscle itself) can determine the efficiency of muscle regeneration. Because this is not accounted for by genetic differences between the bone marrow–derived cells from the two strains, it seems most likely that some factor (possibly blood borne) in the SJL/J host mice affects leukocytes so that they are in a “more activated” state. Earlier experiments with minced muscle autografts in young and old mice showed that impaired macrophage function in old (compared with young) hosts prevented the removal of necrotic tissue and hence new muscle formation, and this inflammatory cell defect was clearly linked to the hormonal status of the host. With respect to the related situation in old and young hosts, it seems likely that inflammatory cells might generally be “less active” in the old host environment, compared with young animals. This idea is supported by strong evidence that an age-related decline in macrophage activity contributes to the slower healing of wounds in old mice. There are a wide range of potential factors that might account for the variation in the “state of activation” of circulating leukocytes; these include hormones and cytokines and the capacity of the cells to respond to them. Before discussing these signals it is pertinent to review the question of whether the number and proliferative potential of satellite cells show any decrease with age.

**Age-related Decline in Satellite Cells?**

Although the relative and absolute proportions of satellite cells to muscle nuclei are affected by innervation and decrease from birth to maturity in rodents, there is little further decrease between muscles of adult and old animals (see ref. 53). The question then arises as to whether the satellite cells lose their capacity to proliferate in old animals? The answer to this question relies in part upon knowing the extent of satellite proliferation in normal uninjured adult and aging muscle. Measurements of the proliferative capacity of human satellite cells indicate that the population of satellite cells undergoes considerable proliferation during the first two decades of life when muscles are growing, but that after this time the population is constant with little or no replication into old age (86 years). Thus it appears that a similar proliferative capacity might be expected for satellite cells from adult and old muscles in response to damage.

Information about the turnover of myonuclei would also indirectly provide information about the proliferation of satellite cells throughout the life of a myofiber. Until recently it was not possible to determine whether there was any turnover of myonuclei within undamaged adult muscle fibers, or if the same myonuclei persisted throughout the life of an individual. This can now be investigated by measuring the length of telomeres
(TTAGGG repeats located at the ends of eukaryotic chromosomes), which are known to decrease with proliferation and are used as an indicator of cellular aging.\(^5\) It has been shown that 86 bp of telomeric DNA is lost with each round of human satellite cell replication in culture.\(^6\) A comparison of telomere restriction fragment (TRF) lengths of myonuclei from young, adult, and old human muscle (9 months to 86 years) showed no significant decrease in the mean TRF length from birth until old age,\(^6\) indicating a tremendous stability of these myonuclei over time, which, in turn, reinforces the idea that satellite cells are essentially quiescent and have minimal turnover in normal uninjured adult muscles. However, comparison of the minimal values of TRF identified a very small increase of 13 bp per year, showing that there is actually a very small turnover of muscle nuclei throughout the life of the myofibers.\(^6\) These elegant studies therefore indicate that there must be some proliferation and fusion of satellite cells throughout life, albeit at an extremely low rate. The simplest explanation is that in adult muscle this occurs sporadically in response to hypertrophy or accidental muscle damage,\(^1\) although it might possibly reflect an extremely low endogenous level of myonuclei turnover. By contrast, a dramatic decrease in mean TRF length is seen in muscle from patients with Duchenne's muscular dystrophy where the muscle is subjected to repeated cycles of necrosis and regeneration.\(^6\)

Tissue culture studies confirm the in vivo observations that satellite cells from old muscle have the capacity to replicate\(^6\) and that the rate of proliferation is not decreased with age.\(^6\) However, tissue cultured muscle cells from old rats consistently have an increased “lag phase” before the onset of replication.\(^4\) In conclusion, it appears that the number and proliferative capacity of satellite cells is not impaired in old (compared with adult) muscle, although the response of these cells may be slower for a range of reasons.

It has often been proposed that the replicative potential of satellite cells may be exhausted by repeated cycles of regeneration in diseases such as muscular dystrophy,\(^4\) and a similar situation might arise theoretically after repeated bouts of extreme exercise over a lifetime.\(^4\) Although in vivo studies indicate that dystrophic muscles retain the capacity to regenerate after experimental injury,\(^4\) tissue culture studies generally support the idea of a loss of proliferative potential of satellite cells from old dystrophic muscles,\(^4\) (for more refs., see refs. 38 and 62). Some caution must be exercised in the interpretation of tissue culture studies of cells from old and young muscles, as there may be difficulties in extracting satellite cells from muscles of different ages and pathologies;\(^38\) for example, it has been estimated that less than 0.01% of myogenic cells are normally extracted from adult muscle under standard procedures.\(^7\) Furthermore the environment from which the cells are extracted may affect their ability to respond under standard tissue culture conditions optimized for growth of myogenic cells from young or adult muscle.\(^38,73\)

**Genetics**

The influence of genetics adds another layer of complexity to the effects of aging. For example, new muscle formation in minced muscle grafts is far more effective in SJL/J than in BALB/c mice,\(^44\) and, in crush-injured muscles, superior regeneration is seen in SJL/J mice and is associated with twice the number of inflammatory cells at three
Inherent differences in myogenicity between these two strains is also demonstrated in tissue culture, where myoblasts from SJL/J mice show an earlier onset of expression of MyoD and myogenin, larger and more frequent myotubes, and a lower dependency on the ECM substrate in comparison with BALB/c myoblasts. So genetics are yet another factor that must be taken into consideration from the perspective of both the host and muscle-related factors.

THE SIGNALS

Vast numbers of growth factors (e.g., fibroblast growth factors (FGF), platelet-derived growth factor-BB (PDGF-BB), IGF, transforming growth factor-β (TGF-β)) have been shown to affect the proliferation and fusion of myoblasts under tissue culture conditions (reviewed in refs. 14, 38, 45, 77–79) and the situation for FGF-2 (widely referred to as basic FGF) is discussed in detail below. Some ECM molecules also have a direct effect on the movement, attachment, proliferation, and fusion of myoblasts: these include the laminins that are associated with the external lamina, specific proteoglycans that are essential for the binding of growth factors like FGF to their receptors (see below), and proteolytic fragments of fibronectin and laminin, which are important chemotactic signals. Recently, there has been additional interest in such factors as hepatocyte growth factor that appears to be an early mitogen for myoblasts, cytokines like interferon-α, interleukin-6, and leukemia inhibitory factor (LIF). A factor produced by crush-injured muscle is of considerable interest, because it has been shown to be a specific and potent mitogen for myoblasts, and recent evidence indicates that this active factor may be hepatocyte growth factor. Bischoff concluded that although this factor can activate satellite cells, a serum factor is required for cells to move through the cell cycle and replicate.

In exercised muscle, a great deal of attention has been focused on IGF-I through its effects on increasing protein (sarcomere) formation. An increase in IGF-I is seen in muscle after acute eccentric exercise, in compensatory hypertrophy, and both IGF-I and another isoform IGF-Ieb increase in response to stretching within two hours. Local production of IGF-I undoubtedly stimulates the growth of postnatal muscle and an increase in muscle mass. Although mRNA for IGF-I is seen in myoblasts and myotubes in injured muscle, and the pattern corresponds closely to that for myoblast proliferation, it is not clear what role IGF-I actually plays as a mitogen during muscle regeneration in response to exercise, compared with its effects on differentiation and protein production.

Studies comparing the response to mitogens of primary muscle cultures from old and young rodents consistently show some decrease in the response of old satellite cells. Mezzogiorno and colleagues investigated the response of old (26 month) mouse muscles to a range of growth factors (FGF, PDGF-BB, IGF-II, ACTH, and LIF) and concluded that there was a generalized reduction in the response to all mitogens tested. Of particular interest was the demonstration that the production of paracrine factors was very different between old and young muscle cells, and they proposed that this led to differences in the local environment in vivo that probably played a major role in the response of young compared to old muscle cells. Age-related differences were also seen among cultured satellite cells from 3-, 12-, and 24-month-old rats, with respect to the number and affinity of
receptors for IGF-II associated with a delayed onset of proliferation in old cells (although the proliferation rates were similar). A delayed response was similarly seen to mitogens from crushed muscle and to FGF-2. The delayed response to FGF-2 with aging satellite cells is discussed in more detail below.

All of these growth factors and ECM molecules must interact in the complex in vivo environment. Many growth factors such as the FGF and TGF-β also stimulate angiogenesis. Many others, including proteolytic fragments of ECM molecules, stimulate the chemotaxis of inflammatory cells and myoblasts. These growth factors and ECM molecules are produced by a wide variety of cells, including myoblasts, fibroblasts, endothelial cells, resident macrophages, dendritic cells, and infiltrating leukocytes. Of the infiltrating leukocytes, it is widely recognized that macrophages play a particularly important role in muscle regeneration.

**Role of Leukocytes**

When muscle is damaged, PML accumulate very rapidly (within minutes) at the injury site; they predominate initially but are largely replaced by macrophages by 24 hours after crush injury. Rapid evascularization of PML in response to chemokines produced by tissue damage has been widely studied and is a very important event in general tissue repair. Tissue culture studies of chemotaxis in muscle and other tissues show that the PML produce soluble factors that chemoattract macrophages to the damage site (see ref. 82). However, the soluble factors produced by PML do not chemoattract myoblasts. Large numbers of platelets may also be present after severe trauma, and they produce many factors (e.g., PDGFs) that facilitate wound repair.

Macrophages, which predominate during skeletal muscle regeneration, are essential for the effective removal of necrotic tissue and produce a vast array of growth factors and enzymes that influence many aspects of the regenerative process, including angiogenesis; the ECM environment; and the chemotaxis, proliferation, and differentiation of myoblasts. There is also evidence that damaged myofibers themselves (in the absence of circulating leukocytes) produce chemotactic signals that attract both macrophages and myoblasts to the injury site.

Thus, in order to evaluate the real importance of various growth factors and ECM molecules during myogenesis, it is essential to assess the effects of them in the complex in vivo environment. There have been remarkably few instances of such in vivo studies in postnatal regenerating skeletal muscles.

**Fibroblast Growth Factors**

It is well documented from tissue culture studies that FGF-2 (previously known as bFGF) is one of the most potent mitogens for myoblasts, and it would appear to play a critical role during myogenesis in developing muscles. On the basis of these data, and the correlation between immunohistochemical studies showing high FGF-2 expression in situations of good muscle regeneration, it was considered that exogenous administration of FGF-2 might enhance new muscle formation, particularly in BALB/c mice where regeneration is usually poor. However, FGF-2 administered in vivo by various
regimes (by injection ± heparin, in hydron or elvax implants) to experimentally injured, denervated, or dystrophic muscle had no effect on myoblast proliferation or the overall histological appearance. The failure of exogenous FGF-2 to enhance the regenerative response indicates that availability of FGF-2 may not normally be the limiting factor in vivo; instead, the cellular responses may be determined by the expression of specific FGF-2 receptors and associated heparan sulphate proteoglycans that regulate the binding of FGF-2.

It is now recognized that proteoglycans sequester heparin-binding growth factors close to cell surfaces and are able to protect them from proteolytic degradation. They are an essential prerequisite for the binding of such factors to their high-affinity cell surface and signal-transducing receptors. Integral membrane species of heparan sulphate molecules, which regulate FGF activity, are known as syndecans. Of particular interest to muscle repair after injury is the report that cellular infiltrates in wounds release a peptide that induces mammalian cells to express syndecans as part of the repair process. If such cellular infiltrates are reduced in old hosts, this could affect the production of syndecans, the speed of response to FGF-2, and hence the onset of satellite cell proliferation and regeneration.

Tissue culture studies on satellite cells show binding of FGF-2 at 18 h (the earliest time examined) and at 42 h postplating in primary cultures from 4-week- and 9-month-old rats, respectively. This correlates with the delayed entry into the cell cycle and the delayed response to FGF-2 seen in satellite cells from old rats. The results suggest that expression of functional FGF receptors on satellite cells may represent an important step in the activation of quiescent satellite cells. An earlier study, which examined the response of young and old muscle in tissue culture to FGF-2, reported no differences in the pattern of myoblast proliferation. However, this study did not look at the onset of the response, and this is probably a critical factor in vivo. Although other factors have been tested and no age-related differences have been observed (reviewed by ref. 45), it is probable that the precise timing of the onset of myoblast activation and replication was not the focus of these studies. Unfortunately, in tissue culture studies it is not possible to study cells prior to about 12 h postplating, as the cells have not fully attached; thus observations on quiescent (time 0) satellite cells and the early phases of activation are not possible.

Other in Vivo Studies

Earlier studies with daily intramuscular injections of the synthetic corticosteroid dexamethasone (1 µg/Kg to 100 µg/Kg) showed no improvement in muscle regeneration after crush injury in BALB/c mice, although tissue culture studies report a stimulation of myoblast proliferation at these low doses. The effect of the cytokine, interferon-α (IFN-α) was also studied in vivo, as interferons are well-known regulators of cellular events and there is conflicting evidence regarding the effects on stimulating myoblast proliferation and fusion in culture: daily intramuscular injections of IFN-α (2.25 × 10^3IU/dose) showed impaired regeneration in SJL/J mice with persisting necrotic tissue, reduced myotube formation, and increased fibrosis at 10 days after crush injury. In contrast with these studies, in vivo administration of LIF is reported to enhance muscle regeneration. The addition of extra macrophages also improves muscle regeneration in vivo, as does the addition of extract from crushed muscles, supporting the idea that regeneration can be
assisted by the exogenous administration of various factors. Anabolic effects of exogenous IGF-I have been demonstrated in dystrophic muscles, although this is probably due largely to a reduction in protein degradation. A further example of exogenous administration of a factor is studies with the thyroid hormone, triiodothyronine, which increased the severity of the dystrophy particularly in younger mdx mice, probably due to metabolic effects and a modulation of myosin synthesis. Mouse strains with inherited defects in genes for specific growth factors and ECM molecules, and engineered “null mutant mice” that lack selected genes, provide many ready opportunities to assess the importance of such factors on exercise, regeneration, and aging in vivo.

CONCLUSIONS

Older muscle generally has a very good capacity for myoblast proliferation and fusion, and hence new muscle formation, although this is slightly less efficient than in younger hosts. It seems likely that optimal cytokine and hormonal production declines with age (and this is also affected by exercise, diet, immune status, and genetics), that such systemic blood-borne factors in the host environment are particularly critical for determining the efficiency of muscle repair in old animals, and that this may be mainly by an effect on the immune response. This is good news. If the factors involved can be identified, this should enable systemic manipulation of the host, possibly by administration of exogenous factors, to enhance muscle repair in old subjects. (The problem of effective reinnervation of regenerated muscle in old hosts is another issue). Clearly other factors intrinsic to the skeletal muscle itself, including changes in the ECM, vascularity, and the expression of growth factors and particularly their receptors by satellite cells, can also contribute to the less efficient regeneration generally seen in old hosts. However, these intrinsic parameters are less readily manipulated.

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