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# Satellite cell depletion in degenerative skeletal muscle

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**Adult skeletal muscle has the striking ability to repair and regenerate itself after injury. This would not be possible without satellite cells, a subpopulation of cells existing at the margin of the myofiber. Under most conditions, satellite cells are quiescent, but they are activated in response to trauma, enabling them to guide skeletal muscle regeneration. In degenerative skeletal muscle states, including motor nerve denervation, advanced age, atrophy secondary to deconditioning or immobilization, and Duchenne muscular dystrophy, satellite cell numbers and proliferative potential significantly decrease, contributing to a diminution of skeletal muscle's regenerative capacity and contractility. This review will highlight the fate of satellite cells in several degenerative conditions involving skeletal muscle, and will attempt to gauge the relative contributions of apoptosis, senescence, impaired proliferative potential, and host factors to satellite cell dysfunction.**

*Keywords:* apoptosis; programmed cell death; sarcopenia; senescence.

## Introduction

Adult skeletal muscle demonstrates the remarkable capacity to adapt to trauma and injury. The processes by which these physiologic adaptations occur are largely attributed to satellite cells, a distinct population of cells at the periphery of the myofiber, first described in 1961.[1] Satellite cells are the myogenic cells responsible for postnatal muscle growth, as well as for the regeneration and repair of injured muscle. Under normal circumstances, satellite cells are quiescent, but in response to stress, they are activated, prompting multiple rounds of proliferation and the expression of myogenic markers.[2] Some satellite cells fuse with one another to form myotubes, whereas others serve as bridges between myofibers, aiding in their regeneration.[3,4]

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Although the exact quantity of satellite cells within skeletal muscle is dependent on muscle fiber type, animal age, and species,[5] the number of quiescent satellite cells essentially remains stable within adults over multiple cycles of injury and regeneration.[6] The absolute number of satellite cells decreases with pathologic states, including Duchenne muscular dystrophy and chronic muscle denervation,[7–9] as well as with normal physiologic decline, such as the onset of advanced age.[10] In addition, satellite cell doubling potential has been demonstrated to be markedly diminished in Duchenne muscular dystrophy,[11] experimental models of unloading-induced skeletal muscle atrophy,[12,13] and old age.[14] With these conditions, skeletal muscle contractile function significantly worsens. It is conceivable that satellite cell depletion promotes this, as the ability to form new myofibers or repair existing ones is markedly impaired.

## Satellite cell depletion secondary to aging

Numerous investigators have demonstrated that, with advancing age, skeletal muscle regenerative capacity diminishes.[15–17] One popular explanation for this is that a decrease in both satellite cell numbers and proliferative capacity leads to a diminution in skeletal muscle's ability to regenerate. Rodent models support this theory, as a significant decrease in the satellite cell population, from 4.6% of all cells at 8 months of age to 2.4% at 30 months of age, has been found in the murine soleus muscle.[10] In addition, a substantial impairment in the proliferative potential, or the number of doublings cultured myoblasts can undergo exists with increasing age.[14] Human skeletal muscle, however, does not demonstrate the same decline in satellite cell numbers with increasing age.[18] In addition, humans fail to demonstrate a difference in the proliferative potential of satellite cells derived from children aged 9 years and those isolated from adults greater than 60 years of age.[19] Thus, it is apparent that the impaired skeletal muscle regenerative response seen with aging in humans likely results from more factors than just declining satellite cell numbers and proliferative capacity.

Apoptosis is a candidate mechanism for satellite cell depletion in aged animals after injury, thereby contributing to an impaired skeletal muscle regenerative response. Satellite cells derived from young and adult rodents, and subsequently expanded *in vitro*, demonstrate significantly diminished percentages of cells with activated caspases and with fragmented DNA as demonstrated by terminal deoxynucleotydil transferase-mediated dUTP nick end labeling (TUNEL) compared to those from old animals after being subjected to a pro-apoptotic milieu via incubation with Tumor Necrosis Factor-$\alpha$ and Actinomycin D.[20] In addition, after exposure to the same pro-apoptotic milieu, satellite cells derived from young and adult rats demonstrated significantly greater expression of the anti-apoptotic protein bcl-2 compared to those derived from old animals.[20] These data suggest that aging increases the satellite cell susceptibility to apoptosis. In old muscle, apoptosis may play a causative role in the depletion of satellite cells, thereby impairing the regenerative response to injury.

Factors within the host environment also affect the regenerative capacity of skeletal muscle, as well as the satellite cell response to injury. Cross-transplantation of hindlimb skeletal muscle between 4-month-old and 24-month-old rats reveals that skeletal muscle from older rodents demonstrates no greater deficit in force-generating capacity as compared to that from young animals after transplant-induced and bupivicaine-induced injury; this is not seen when the muscle is left in its native environment.[21,22] In addition, old animals are less capable of mounting an immune response, resulting in a decrease in circulating inflammatory factors and growth factors,[23] including Insulin-like Growth Factor-I (IGF-I).[24] Inflammatory factors, which include macrophages and associated cytokines, are critical for normal satellite cell activation and proliferation.[2] Furthermore, IGF-I has been demonstrated to restore skeletal muscle mass and satellite cell proliferative potential after multiple cycles of hindlimb loading and unloading,[25] as well as to protect satellite cells from apoptosis.[26] It is thus evident that many variables, including satellite cell senescence, diminishing satellite cell numbers, increased satellite cell susceptibility to apoptosis, and changing factors within the local environment, all contribute to the impaired regenerative capacity of aged skeletal muscle.

## Satellite cell depletion secondary to skeletal muscle denervation

Peripheral motor nerve injury results in both skeletal muscle atrophy and a permanent deficit in contractile function.[27,28] In response to motor nerve transection, satellite cells undergo an initial phase of rapid proliferation.[9,29] A prolonged period of denervation, however, leads to dramatic drop in satellite cell numbers, so that satellite cells represent approximately 1% of all cells present within the skeletal muscle cross-section, down from about 9% in the acute period after denervation.[29] This phenomenon begins to occur in the interval 7 weeks to 20 weeks postdenervation.[9] Chronic skeletal muscle denervation has detrimental repercussions, as permanent disability results after virtually all such injuries, even if neuronal sprouting and regeneration have occurred.[30]

The progressive decline in the satellite cell population may well be the result of programmed satellite cell death. As early as 2 months after motor nerve transection, a marked increase in the numbers of nuclei with hypercondensed chromatin and fragmented DNA is present diffusely throughout the muscle cross-section.[31] Although most of these apoptotic cells likely represent myonuclei, it is difficult to assess for satellite cell apoptosis *in vivo* secondary to their relative dearth within denervated skeletal muscle.[29] Satellite cells derived from skeletal muscle 6 and 10 weeks after motor nerve transaction and subsequently exposed to an *in vitro* proapoptotic milieu demonstrate a more than twofold increase in percentages of cells with fragmented DNA and with activated caspases compared to those isolated from innervated, age-matched control muscle.[32] Although the mechanisms responsible for the increased satellite cell susceptibility to apoptosis have not been elucidated, it is reasonable to speculate that the signals triggering programmed cell death may be related to either the loss of neuroelectrical stimulation or neurotrophic factors. Interruption of the close relationship between a nerve and its peripheral target organ leads to cell death in many different tissues during development.[33,34] Although the complex interaction between mature motor neurons and skeletal muscle is appreciated only at a rudimentary level, end-organ cell death is an almost certain occurrence after peripheral nerve injury.

## Satellite cell depletion with skeletal muscle atrophy

Sarcopenia, or a reduction in muscle mass, can result from a number of conditions, some of which are pathologic, such as denervation, but most of which are physiologic, including prolonged immobilization, malnutrition and illness.[35] Clinically, sarcopenia can be seen in persons with prolonged hospitalizations secondary to prolonged illness or physical frailty. Because of skeletal muscle contractile dysfunction, these people have an inability to perform needed activities of daily living, leading to an obviously diminished quality of life.[36] In the laboratory, hindlimb suspension or skeletal muscle immobilization can be used to induce physiologic atrophy in rodents.[37]

In adolescent rats, hindlimb immobilization irreversibly diminishes the numbers of satellite cells and impairs satellite cell proliferative potential within both predominantly slow-twitch and fast-twitch muscles.[12,38] In contrast, although a decline in satellite cell content is seen with immobilization of the adult rat hindlimb, remobilization promotes a complete recovery of skeletal muscle mass and regeneration of myofibers.[39,40] This suggests that, unlike the satellite cells of adolescent rodents, the satellite cells of adult animals can activate and proliferate to promote recovery of sarcopenic muscle. In old rodents, a single episode of hindlimb immobilization lasting 10 days leads to a significant loss in skeletal muscle mass and satellite cell proliferative potential, even when the animal has been permitted 9 weeks recovery time.[25] Hence, although satellite cell proliferative potential is restored in remobilized skeletal muscle from adult animals, the same phenomenon is not observed in satellite cells from adolescent and geriatric animals, both at the extremes of development.

When IGF-I is infused into the gastrocnemius of old animals after multiple bouts of atrophy induction by hindlimb immobilization, the muscle recovers mass and increases its total protein content. Furthermore, satellite cells recovered from the muscle experience a significant increase in proliferative potential.[25] This observation suggests that innate deficiencies of satellite cells are not the sole explanation for satellite cell dysfunction in aged animals and that host factors likely play a crucial role in satellite cell replicative senescence. The relative contributions of each factor have yet to be determined.

## Satellite cell depletion with exercise

Short bursts of skeletal muscle contraction against high resistance stimulate muscle hypertrophy.[41] Resistance training, which represents minor skeletal muscle trauma, generates muscle hypertrophy through the activation of satellite cells, subsequently followed by satellite cell proliferation, chemotaxis to damaged myofibers, and the fusion of satellite cells to existing myofibers, thereby promoting muscle growth.[5] Satellite cell chemotaxis requires the presence of an intact basal lamina. With resistance training, or limited myotrauma, no disruption of the basal lamina occurs, permitting satellite cells to migrate from their normal position under the basal lamina to the site of injury.[42]

In response to exercise-induced trauma to skeletal muscle, an acute inflammatory reaction is evoked, resulting in a massive influx of macrophages into the injured muscle. Macrophages are critical in the repair of skeletal muscle, as they release cytokines and growth factors that control satellite cell activation and proliferation.[43] This is confirmed when the appropriate macrophage influx fails to occur, as a failure of appropriate muscle regeneration is seen. Similarly, when the macrophage response is intensified, satellite cell activation is enhanced.[44]

Resistance training promotes macrophage release of multiple growth factors, including IGF-I, that promote satellite cell activation, proliferation, and fusion, ultimately leading to muscle hypertrophy.[45] Additional growth factors and cytokines may also promote satellite cell activation, thereby playing a role in the hypertrophic response of skeletal muscle to resistance training, including Hepatocyte Growth Factor, members of the Fibroblast Growth Factor family, and Leukocyte Inhibitory Factor;[46–48] others, including Transforming Growth Factor-$\beta$ and interleukin-6, may inhibit satellite cell proliferation and differentiation.[49,50] Although the complex interplay among the various growth factors and cytokines in not understood fully, the primary consequence of repeated, limited myotrauma is satellite cell activation promoting muscle hypertrophy, and ultimately, skeletal muscle with increased force generating capacity.[2]

All movement represents a series of coordinated muscle contractions, in which skeletal muscle either shortens, remains constant in length, or stretches while contracting.[51] Significant injury to skeletal muscle fibers is most likely during those contractions that occur while activated muscle fibers are stretched, known as lengthening contractions.[52] Although the mechanism by which lengthening contractions produce myofiber injury is uncertain, the initial injury is mechanical and focal within small clusters of sarcomeres.[53] This initiates a series of events, ultimately culminating in secondary injury that is most severe days after the initial insult.[52] At the peak of injury, as well as having obvious morphologic damage, skeletal muscles from both young and old animals manifest a deficit in contractile force generating capacity, though the injury is most severe in the old animals.[54,55] In addition to sustaining greater injury compared to that from younger animals, skeletal muscle from old animals demonstrates an impaired ability to recover force generating capacity after contraction-induced injury.[16] Although little data exists regarding the ultimate fate of the satellite cell population in this scenario, it is reasonable to speculate that either failure of satellite cell activation or loss of satellite cells is responsible for the impaired recovery of skeletal muscle after contraction-induced injury in the aged population. Unpublished work from our laboratory reveals that, compared to satellite cells derived from mature adult rats, a significantly greater percentage of satellite cells derived from aged rats have activated caspases and have fragmented DNA as demonstrated by TUNEL after being subjected to an *in vitro* proapoptotic milieu (manuscript in preparation; data presented at the 48th annual Plastic Surgery Research Council, April 25, 2003, Las Vegas). These findings suggest that increased satellite

cell apoptosis contributes to the impaired regenerative capacity of skeletal muscle from old animals after contraction-induced injury.

## Satellite cell depletion with myopathy

The majority of myopathies occur as the result of a mutation affecting cytoskeletal proteins in muscle. Duchenne muscular dystrophy (DMD), an X-linked recessive disease affecting primarily skeletal and cardiac muscle, is the most common deadly genetic disorder in children, affecting nearly 1 in 3500 males worldwide.[56] Cardiac and skeletal muscles lack the protein dystrophin, which serves as a link between cytoskeletal actin and the extracellular matrix. This connection is essential for maintaining the integrity of muscle cell membranes and its absence results in extremely fragile myofibers.[57] The repeated mechanical stress brought on by recurring contractions results in progressive muscle degeneration. The satellite cell response to the ongoing trauma is to replenish injured skeletal muscle with myofibers lacking dystrophin, resulting in multiple cycles of muscle degeneration and regeneration. Ultimately, this exhausts the satellite cell population.[8,58]

Patients with DMD begin to manifest symptoms at 4 to 5 years of age, with most boys losing the ability to walk between 8 to 10 years of age. Progressive respiratory insufficiency begins early in the second decade of life, with death usually occurring in the late teens or early twenties as the result of respiratory or cardiac failure.[59] Using loss of telomere length to quantify the intensity of muscle cell turnover, it has been demonstrated that skeletal muscles derived from dystrophic patients as young as 4 years old, the age at which clinical symptoms first become apparent, have already undergone extensive regeneration; the rate of telomere loss is 14 times greater than that observed in controls.[60] Consistent with this, the proliferative potential of satellite cells derived from a 9-year-old dystrophic patient is only one-third of that of satellite cells harvested from age-matched controls.[19] Together, these findings suggest that the diminution of dystrophic skeletal muscle regenerative capacity is largely the result of early satellite cell senescence induced by accelerated cycles of muscle degeneration and satellite cell proliferation.

The host environment within dystrophic skeletal muscle may also contribute to the impaired proliferative potential of satellite cells. Within patients with DMD, fibroblasts release increased amounts of IGF binding proteins, which sequester IGFs and limit their bioavailability to satellite cells.[61] As both IGF-I and IGF-II are vital in maintaining satellite cell proliferation and differentiation,[62,63] it is probable that limiting their availability to satellite cells promotes senescence. The IGFs also likely play a role in the prevention of programmed satellite cell death within skeletal muscle, as they have been demonstrated to do *in vitro* with myoblasts.[26,64]

In the *mdx* mouse, which lacks dystrophin and provides an animal model for the study of DMD, degeneration of dystrophic skeletal muscle largely is the result of necrotic cell death. Multiple authors have demonstrated, however, that apoptosis precedes necrosis in some dystrophin-deficient myofibers of *mdx* mice, both by TUNEL and DNA ladder analysis.[65–67] Similar findings have been observed in the skeletal muscle of children with DMD, with as much as 10% of non-necrotic muscle fibers demonstrating evidence of *in situ* DNA fragmentation.[68] In addition to myofibers, satellite cells and macrophages with evidence of DNA fragmentation, suggestive of apoptosis, have been identified within the skeletal muscle of patients with DMD.[69] Therefore, although satellite cell senescence secondary to multiple cycles of muscle degeneration and satellite cell proliferation likely plays the predominant role, increased rates of satellite cell apoptosis may also promote impaired skeletal muscle regeneration in patients with DMD.

## Future directions

Satellite cells are essential for the normal growth, repair, and regeneration of adult skeletal muscle. After physiologic degeneration, such as occurs with aging, as well as with pathologic states, including denervation atrophy and DMD, satellite cell dysfunction, whether the result of diminished activation, senescence, or increased apoptosis, is the principal causes of skeletal muscle's impaired regenerative capacity. What is uncertain, however, is whether the restoration of satellite cell function can ameliorate the contractile dysfunction seen in enervated or diseased skeletal muscle. Although no method yet exists to overcome normal satellite cell senescence or impaired satellite cell activation, pharmacologic inhibitors of critical second messenger systems exist to block apoptosis. It is conceivable that, in the future, therapeutic modalities inhibiting apoptotic processes may have a role in minimizing the loss of satellite cells that occurs with physiologic and pathologic processes. This may prevent satellite cell depletion and, because of their requisite role in regenerating and restoring injured muscle, enhance the regenerative capacity of skeletal muscle.

### References

1. Mauro A. Satellite cell of skeletal muscle fibers. *J Biophys Biochem Cytol* 1961; 9: 493–495.
2. Hawke TJ, Garry DJ. Myogenic satellite cells: Physiology to molecular biology. *J Appl Physiol* 2001; 91: 534–551.

3. Lu DX, Huang SK, Carlson BM. Electron microscopic study of long-term denervated rat skeletal muscle. *Anat Rec* 1997; **248**: 355–365.
4. Bischoff R. The satellite cell and muscle regeneration. In: Engel AG and Frazini-Armstrong C, ed. *Myology*. New York: McGraw-Hill 1994: 97–118.
5. Schultz E, McCormick KM. Skeletal muscle satellite cells. *Rev Physiol Biochem Pharmacol* 1994; **123**: 213–257.
6. Gibson MC, Schultz E. Age-related differences in absolute numbers of skeletal muscle satellite cells. *Muscle Nerve* 1983; **6**: 574–580.
7. Webster C, Blau HM. Accelerated age-related decline in replicative life-span of Duchenne muscular dystrophy myoblasts: Implications for cell and gene therapy. *Somatic Cell Mol Genet* 1990; **16**: 557–565.
8. Heslop L, Morgan JE, Partridge TA. Evidence for a myogenic stem cell that is exhausted in dystrophic muscle. *J Cell Sci* 2000; **113**: 2299–2308.
9. de Castro Rodrigues A, Schmalbruch H. Satellite cells and myonuclei in long-term denervated rat muscles. *Anat Rec* 1995; **243**: 430–437.
10. Snow MH. The effects of aging on satellite cells in skeletal muscles of mice and rats. *Cell Tissue Res* 1977; **185**: 399–408.
11. Schultz E, Jaryszak DL. Effects of skeletal muscle regeneration on the proliferation potential of satellite cells. *Mech Ageing Dev* 1985; **30**: 63–72.
12. Darr KC, Schultz E. Hindlimb suspension suppresses muscle growth and satellite cell proliferation. *J Appl Physiol* 1989; **67**: 1827–1834.
13. Schultz E, Darr KC, Marius A. Acute effects of hindlimb unweighting on satellite cells of growing skeletal muscle. *J Appl Physiol* 1994; **76**: 266–270.
14. Schultz E, Lipton BH. Skeletal muscle satellite cells: Changes in proliferation potential as a function of age. *Mech Ageing Dev* 1982; **20**: 377–383.
15. Carlson BM, Faulkner JA. Muscle regeneration in young and old rats: Effects of motor nerve transection with and without marcaine treatment. *J Gerontol A Biol Sci Med Sci* 1998; **53**: B52–B57.
16. Brooks SV, Faulkner, JA. Contraction-induced injury: Recovery of skeletal muscles in young and old mice. *Am J Physiol* 1990; **258**: C436–C442.
17. Grounds MD. Age-associated changes in the response of skeletal muscle to exercise and regeneration. *Ann NY Acad Sci* 1998; **854**: 78–91.
18. Roth SM, Martel GF, Ivey FM, *et al*. Skeletal muscle satellite cell populations in healthy young and older men and women. *Anat Rec* 2000; **260**: 351–358.
19. Renault V, Piron-Hamelin G, Forestier C, *et al*. Skeletal muscle regeneration and the mitotic clock. *Exp Gerontol* 2000; **35**: 711–719.
20. Jejurikar SS, Cederna PS, Marcelo CL, Kuzon WM. Aging promotes satellite cell apoptosis (abstract). *J Am Coll Surg* 2002; **195**: S46.
21. Carlson BM, Faulkner JA. Muscle transplantation between young and old rats: Age of host determines recovery. *Am J Physiol Cell Physiol* 1989; **256**: C1262–C1266.
22. Carlson BM, Faulkner JA. The regeneration of noninnervated muscle grafts and marcaine-treated muscles in young and old rats. *J Gerontol A Biol Sci Med Sci* 1996; **51**: B43–B49.
23. Danon D, Kowatch MA, Roth GS. Promotion of wound repair in old mice by local injection of macrophages. *Proc Natl Acad Sci USA* 1989; **86**: 2018–2020.
24. Ullman M, Ullman A, Sommerland H, Skottner A, Oldfors A. Effects of growth hormone on muscle regeneration and IGF-I concentration in old rats. *Acta Physiol Scand* 1990; **140**: 521–525.
25. Chakravarthy MV, Davis BS, Booth FW. IGF-I restores satellite cell proliferative potential in immobilized old skeletal muscle. *J Appl Physiol* 2000; **89**: 1365–1379.
26. Napier JR, Thomas MF, Sharma M, Hodgkinson SC, Bass JJ. Insulin-like growth factor-I protects myoblasts from apoptosis but requires other factors to stimulate proliferation. *J Endocrinology* 1999; **163**: 63–68.
27. Sunderland S, Ray LJ. Denervation changes in mammalian striated muscle. *J Neurology Neurosurg Psych* 1950; **13**: 159–177.
28. Tower S. The reaction of muscle to denervation. *Physiol Rev* 1939; **19**: 1–48.
29. Viguie CA, Lu DX, Huang SK, Rengen H, Carlson BM. Quantitative study of the effects of long-term denervation on the extensor digitorum longus muscle of the rat. *Anat Rec* 1997; **248**: 346–354
30. Sunderland S. Traumatized nerves, roots and ganglia: Musculoskeletal factors and neuropathological consequences. In: Korr IM, ed. *The Neurobiologic Mechanisms in Manipulative Therapy*. New York: Plenum 1978: 137–166.
31. Borisov AB, Carlson BM. Cell death in denervated skeletal muscle is distinct from classical apoptosis. *Anat Rec* 2000; **258**: 305–318.
32. Jejurikar SS, Marcelo CL, Kuzon WM. Skeletal muscle denervation increases satellite cell susceptibility to apoptosis. *Plast Recons Surg* 2001; **110**: 160–168.
33. Raff MC, Barres BA, Burne JF, Coles HS, Ishizaki Y, Jacobson MD. Programmed cell death and the control of cell survival: Lessons from the nervous system. *Science* 1993; **262**: 695–700.
34. Oppenheim RW. Cell death during development of the nervous system. *Ann Rev Neurosci* 1991; **14**: 453–501.
35. Grounds MD. Muscle regeneration: Molecular aspects and therapeutic implications. *Curr Opin Neurol* 1999; **12**: 535–543.
36. Winograd CH, Gerety MB, Chung M, Goldstein MK, Dominguez F, Vallone R. Screening for frailty: Criteria and predictors of outcome. *J Am Geriatr Soc* 1991; **39**: 778–784.
37. Fitts RH, Metzger JM, Riley DA, Unsworth BR. Models of disuse: A comparison of hindlimb suspension and immobilization. *J Appl Physiol* 1986; **60**: 1946–1953.
38. Mozdziak PE, Pulvermacher PM, Schultz E. Unloading of juvenile muscle results in a reduced muscle size 9 weeks after reloading. *J Appl Physiol* 2000; **88**: 158–164.
39. Booth FW. Regrowth of atrophied skeletal muscle in adult rats after ending immobilization. *J Appl Physiol* 1978; **44**: 225–230.
40. Wanek LJ, Snow MH. Activity-induced fiber regeneration in rat soleus muscle. *Anat Rec* 2000; **258**: 176–185.
41. Rosenblatt JD, Yong D, Parry DJ. Satellite cell activity is required for hypertrophy of overloaded adult rat muscle. *Muscle Nerve* 1994; **17**: 608–613.
42. Schultz E, Jaryszak DL, Valliere CR. Response of satellite cells to focal skeletal muscle injury. *Muscle Nerve* 1985; **8**: 217–222.
43. Nathan CF. Secretory products of macrophages. *J Clin Invest* 1987; **79**: 319–326.
44. Lescaudron L, Peltekian E, Fontaine-Perus J, *et al*. Blood borne macrophages are essential for the triggering of muscle regeneration following muscle transplant. *Neuromuscul Disord* 1999; **9**: 72–80.
45. Vierck J, O'Reilly B, Hossner K, *et al*. Satellite cell regulation following myotrauma caused by resistance exercise. *Cell Biol Int* 2000; **24**: 263–272.
46. Tatsumi R, Anderson JE, Nevoret CJ, Halevy O, Allen RE. HGF/SF is present in normal adult skeletal muscle and is capable of activating satellite cells. *Dev Biol* 1998; **194**: 114–128.

47. Sheehan SM, Allen RE. Skeletal muscle satellite cell proliferation in response to members of the fibroblast growth factor family and hepatocyte growth factor. *J Cell Physiol* 1999; **181**: 499–506.
48. Kurek JB, Bower JJ, Romanella M, Koentgen F, Murphy M, Austin L. The role of leukemia inhibitory factor in skeletal muscle regeneration. *Muscle Nerve* 1997; **20**: 815–822.
49. Martin JF, Li L, Olson EN. Repression of myogenin function by TGF-$\beta$1 is targeted at the basic helix-loop-helix motif and is independent of E2A products. *J Biol Chem* 1992; **267**: 10956–10960.
50. Kami K, Senba E. Localization of leukemia inhibitory factor and interleukin-6 messenger ribonucleic acids in regenerating rat skeletal muscle. *Muscle Nerve* 1998; **21**: 819–822.
51. Brooks SV, Opiteck JA, Faulkner JA. Conditioning of skeletal muscle in adult and old mice for protection from contraction-induced injury. *J Gerontol A Biol Sci* 2001; **56A**: B163–B171.
52. McCully KK, Faulkner JA. Injury to skeletal muscle fibers of mice following lengthening contractions. *J Appl Physiol* 1985; **59**: 119–126.
53. Newham DJ, McPhail G, Mills DR, Edwards RHT. Ultrastructural changes after concentric and eccentric contractions. *J Neurol Sci* 1983; **61**: 109–122.
54. Brooks SV, Faulkner JA. The magnitude of the initial injury induced by stretches of maximally activated muscle fibres of mice and rats increases in old age. *J Physiol (London)* 1996; **497**: 573–580.
55. Faulkner JA, Brooks SV, Zerba E. Muscle atrophy and weakness with aging: Contraction-induced injury as an underlying mechanism. *J Gerontol A Biol Sci Med Sci* 1995; **50A**: 124–129.
56. England SB, Nicholson LV, Johnson MA, *et al.* Very mild muscular dystrophy associated with the deletion of 46% of dystrphin. *Nature* 1990; **343**: 180–182.
57. Rybakova IN, Patel JR, Ervasti JM. The dystrophin complex forms a mechanically strong link between the sarcolemma and costameric actin. *J Cell Biol* 2000; **150**: 1209–1214.
58. Cossu G, Mavilio F. Myogenic stem cells for the therapy of primary myopathies: Wishful thinking or therapeutic perspective? *J Clin Invest* 2000; **105**: 1669–1674.
59. McDonald CM, Abresch RT, Carter GT, *et al*. Profiles of neuromuscular diseases: Duchenne muscular dystrophy. *Am J Phys Med Rehabil* 1995; 74 **(suppl)**: S70–S92.
60. Decary S, Hamida CB, Mouly V, Barbet JP, Hentati F, Butler-Browne GS. Shorter telomeres in dystrophic muscle consistent with extensive regeneration in young children. *Neuromuscul Disord* 2000; **10**: 113–120.
61. Melone MA, Peluso G, Galderisi U, Petillo O, Cotrufo R. Increased expression of IGF-binding protein-5 in Duchenne muscular dystrophy (DMD) fibroblasts correlates with the fibroblast-induced downregulation of DMD myoblast growth: An *in vitro* analysis. *J Cell Physiol* 2000; **185**: 143–153.
62. Doumit ME, Cook DR, Merkel RA. Fibroblast growth factor, epidermal growth factor, insulin-like growth factors, and platelet-derived growth factor-BB stimulate proliferation of clonally derived porcine myogenic satellite cells. *J Cell Physiol* 1993; **157**: 326–332.
63. Haugk KL, Roeder RA, Garber MJ, Schelling GT. Regulation of muscle cell proliferation by extracts from crushed muscle. *J Anim Sci* 1995; **73**: 1972–1981.
64. Smith J, Fowkes G, Schofield PN. Programmed cell death in dystrophic (*mdx*) muscle is inhibited by IGF-II. *Cell Death Differ* 1995; **2**: 243–251.
65. Sandri M, Carraro U, Podhorska-Okolow M, *et al*. Apoptosis, DNA damage and ubiquitin expression in normal and *mdx* muscle fibers after exercise. *FEBS Letters* 1995; **373**: 291–295.
66. Tidball JG, Albrecht DE, Lokensgard BE, Spencer MJ. Apoptosis precedes necrosis of dystrophin-deficient muscle. *J Cell Sci* 1995; **108**: 2197–2204.
67. Matsuda R, Nishikawa A, Tanaka H. Visualization of dystrophic muscle fibers in *mdx* mouse by vital staining with Evens Blue: Evidence of apoptosis in dystrophin-deficient muscle. *J Biochem (Tokyo)* 1995; **118**: 959–964.
68. Tews DS, Goebel HH. DNA-fragmentation and expression of apoptosis-related proteins in muscular dystrophies. *Neuropathol Appl Neurobiol* 1997; **23**: 331–338.
69. Sandri M, Minetti C, Pedemonte M, Carraro U. Apoptotic myonuclei in human Duchenne muscular dystrophy. *Lab Invest* 1998; **78**: 1005–1016.