Denervation and the Aging of Skeletal Muscle

Bruce M. Carlson

Institute of Gerontology and Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, Michigan USA

Abstract
There are many striking similarities in the reactions of mammalian skeletal muscle to denervation and the normal aging process. In both cases, the tissue environment is far more complex than has commonly been assumed, with satellite cell activation and new muscle fiber formation occurring simultaneously with atrophy and degeneration. Some of the changes that take place during aging can be attributed to denervation. During the normal aging process motor axons begin to die, and the muscle fibers that they supply are either temporarily denervated until they are reinnervated by sprouting from other axons, or they may remain permanently denervated and ultimately disappear. During aging cellular and molecular phenomena that are commonly associated with denervation increase with advancing age. This accelerates greatly in the period just before death, reflecting both increased denervation and an overall homeostatic decompensation in which the developmental controls that lend stability to normal mature muscle appear to break down.
Key words: muscle, aging, denervation.

Basic Appl Myol 14(3) 135-139, 2004

Aging mammalian skeletal muscle is characterized by a progressive loss of mass and contractile force [10, 23]. In recent years the term, sarcopenia, has been applied to the extreme loss of muscle mass [35]. The underlying basis for sarcopenia has not been clearly defined, and it will likely prove to be multifactorial. One of the factors that will undoubtedly prove to be significant is the neural support of aging muscle.

Over recent decades it has become clear that as an individual ages the number of motor axons supplying a muscle declines measurably [6, 17, 25, 26, 31, 37]. The loss of alpha-motoneurons is greatest among those that supply fast (type II) muscle fibers [24, 33]. This is followed by motor unit remodeling, in which muscle fibers that have lost their primary innervation may be secondarily reinnervated by sprouts from other axons in the vicinity. Commonly the sprouts arise from slow axons, with the result that there is an expansion of the size and resulting force of slow motor units in the old muscle [26, 32]. If a muscle fiber deprived of its innervation does not become reinnervated, it then undergoes denervation changes and may ultimately disappear.

Whereas over a long period the overall denervation effect in an aging muscle might be quite significant, at a given point in time, denervation changes typically affect only a small proportion of the muscle fibers in a particular muscle. Therefore it is difficult to do quantitative studies on the impact of denervation in normally aging muscle. For a better understanding of the role that denervation plays in muscle aging and its effect on the properties of aging muscle, it is useful to study fully denervated muscle, where it is much easier to relate changes to the lack of innervation [14].

Comparisons between Denervation and Aging Changes in Muscle

Denervation per se produces rapid and profound effects on skeletal muscle, and recent research has shown relatively few differences between the reactions of young adult and old muscle to denervation [5, 15, 19]. Therefore one can extrapolate with some degree of confidence the results of denervation studies on adult muscle to old muscle. For the sake of consistency, this review will concentrate on research conducted on denervated and aging rat muscle. Data obtained from studies on other species will be identified as such.

Gross Tissue-Level Changes.

Studies on the denervated adult rat extensor digitorum longus (EDL) muscle have shown a very rapid loss of both mass (2/3 within a month) and isometric contractile force (95% within a month) until by 4 months after denervation the muscle has stabilized to approximately 25% of its original mass and less than 0.1% of its maximum isometric contractile force [13]. In one of the

few differences from young adult muscle, denervated muscle in 2-year old rats undergoes a slightly slower rate of atrophy (both mass and force)[15].

Loss of mass and force is much slower and much less profound during the normal aging process. Nevertheless, in the strain of Wistar rats used in our laboratory, by 2 years the mass of the EDL muscle is 95% and the maximum force 85% that of young adult control values. In animals close to death (at 32 months for this strain) the mean mass of the EDL muscle had declined to 51% of control values and the maximum force was only 17% of that of adult control muscles [16].

Much of the gross muscle atrophy following denervation is attributable to atrophy of the muscle fibers themselves. During the early months after denervation, differential fiber-type atrophy is seen in some muscles. In the rat EDL, in particular, the type II (fast) muscle fibers undergo a steady atrophy, beginning soon after denervation, whereas the type I (slow) muscle fibers remain little affected for several months, before they also begin a rapid course of atrophy [8, 15, 34]. However, it is important to note that different muscles react quite differently from one another with respect to patterns of atrophy, and it is difficult to generalize from one muscle to another [18]. Aging muscle fibers also undergo a degree of atrophy [11, 12, 31], but the situation is made more complex by the dynamics of motor unit remodeling. In the rat, at least, type I fibers have a smaller mean cross-sectional area than do type II, and when formerly type II fibers become incorporated into type I motor units, the overall mean cross-sectional area of the muscle fiber population is skewed toward a smaller size.

Many studies of aging muscle have concluded that there is a moderate loss of muscle fibers during the aging process [32]. Kaminska et al. [27] reported extensive muscle fiber fragmentation in extremely old rats. Surprisingly after long-term denervation the total number of muscle fibers and the number of muscle fibers per fascicle actually increases despite the massive atrophy [20]. There is also a substantial increase in the number of intrafusal fibers in denervated muscle spindles [Borisov and Carlson, unpublished; 36, 42, 47].

At the tissue level, a profound loss of the microvasculature is accompanied by a substantial build-up of interstitial connective tissue in long-term denervated muscle [9]. In fact, entire fascicles of denervated muscle fibers may not have any capillaries in a given cross-section. Instead the atrophic muscle fibers are encased in thick masses of collagen fibers. In old age, rat muscle is characterized by a slight reduction in the microvasculature and a build-up of interstitial collagen, but these changes do not approach the level seen in denervated muscles.

Satellite Cells

Satellite cells are very responsive to denervation. Within a week, their numbers in the denervated rat EDL have doubled (Rengen and Carlson, unpublished), and by 2 months after denervation the percentage of satellite cells (satellite cells/myonuclei + satellite cells) has increased by a factor of three over control levels although the increase in absolute number is somewhat less because of the loss of some myonuclei [39, 43]. After 2 months of denervation, both the percentage and numbers of satellite cells decline steadily so that by 7 months the percentage has declined to control values, but the absolute numbers are only one third of control values. Quantitation of satellite cells in extremely long-term denervated muscles of rats is complicated by aging changes. Thus, the 0.5% of satellite cells in the 25-month denervated EDL muscle of the rat must be viewed in light of the reduction of satellite cell percentages from 2.9% in 4-month control EDL muscles to 1.4% in the 29-month old EDL [20]. Structurally, satellite cells in long-term denervated muscle show structural signs of activation, as is evidenced by increased amounts of cytoplasm seen in electron micrographs and the formation of long cytoplasmic processes as seen in light micrographs [8, 19, 20, 34].

The percentage of satellite cells declines sharply over the lifespan of the individual, but in rats, the bulk of the decline occurs during the first month after birth [2]. Ouring most of adult life the percentage of satellite cells remains remarkably constant. Only during old age do substantial decreases in satellite cell percentages become apparent [20, 22]. A similar quantitative pattern of satellite cells is seen in human muscle [38].

Occasionally in very old muscle the percentage of satellite cells actually increases, particularly in animals affected with hindlimb neuropathy [16]. This is likely a response of the satellite cells to denervation, since the response of satellite cells in old animals to denervation is remarkably similar to that seen in young adults (19).

Cell and Nuclear Death

Both myonuclear and muscle fiber death are prominent features of the postdenervation landscape [7, 40]. Occurring throughout most of the postdenervation period, nuclear death accounts for the loss of roughly one myonucleus per day from a rat muscle fiber during the first 4 months after denervation [43]. The morphological sequence of myonuclear death is almost identical to that seen in other apoptotic systems, with chromatin condensation, clumping around the periphery of the nucleus, and ultimately fragmentation following a predictable sequence. The question of why some nuclei undergo death while their close neighbors remain apparently perfectly healthy has not yet been resolved. Much more remains to be learned about the local distribution and amounts of pro- and antiapoptotic factors within the denervated muscle fiber.

In addition to myonuclei, entire denervated muscle fibers also die [7, 8]. At the ultrastructural level, muscle fiber death is evidenced not only by the presence of obviously dying fibers, but by the persistence of the empty basal laminae that surround the muscle fibers. At present there is little understanding about the pathway that leads from nuclear death to cellular death in some muscle fibers.

Aging muscle in rats also shows evidence of apoptosis, but its frequency is much less than that seen in denervated muscle. It is difficult to know how much of the fiber death in aging muscle is due to the denervation of some muscle fibers and how much may be due to a direct effect of the aging process.

Neomyogenesis

One of the paradoxical features of denervated muscle is that despite massive atrophy and presence of cell death, large amounts of new muscle fibers concurrently form [8, 20, 41]. There appear to be two phases and types of new muscle fiber formation after denervation in the rat. The first type, which begins between two and three weeks after denervation (before significant cell death has occurred)and is most common during the first and second months, resembles secondary myogenesis in prenatal life and consists of the fusion of activated satellite cells along the surface of existing muscle fibers. The new muscle fibers do not necessarily have the same phenotype as the parent fibers. For example, it is not uncommon for fibers staining immunologically for slow myosin to be found alongside fast muscle fibers.

A second type of neomyogenesis consists of the activation of satellite cells beneath the basal lamina of degenerated muscle fibers, and the morphology of the process closely resembles that of post-traumatic muscle fiber regeneration. In this type of neomyogenesis it is not uncommon for several new muscle fibers to differentiate beneath a single original basal lamina. One unusual feature of new muscle fibers formed in denervated muscle is that they are almost uniformly lacking in accompanying satellite cells [20]. This feature might account for the poor restorative capacity of long-term denervated muscle.

Satellite cell activation and new muscle fiber formation also occur in aging muscle, but to a considerably lesser degree. As is so often the case in old muscle, it is difficult to determine whether the neomyogenesis that does occur is all connected with muscle fibers that have become denervated or whether satellite cells become activated and fuse in innervated fibers, as well.

Gene Expression

In both aging and denervated muscle, the expression of genes that are characteristic of developing or regenerating muscle and are normally quiescent in normal adult muscle occurs. Denervation results in a rapid expression of myogenin and other myogenic regulatory factors [30, 44, 45, 46], with levels of both myogenin RNA and protein levels increasing several-fold within 24 hours after denervation. This precedes the morphological activation of satellite cells. mRNA levels of the other myogenic regulatory factors similarly rise during the first weeks after denervation [1]. Levels of myogenin and MRF4 mRNAs fall thereafter, whereas those of MyoD and Myf-5 remain elevated for a prolonged period.Denervation also results in the expression of the embryonic isoform of myosin, principally in newly forming muscle fibers, but immunocytochemical staining for embryonic myosin has also been seen in parent muscle fibers [8]. Among the subunits of the acetylcholine receptor complex, the γ-subunit, which is normally expressed in the embryonic preinnervated stage of muscle development, is strongly expressed shortly after denervation, but over succeeding months, the levels of this subunit fall markedly [1]. A similar pattern is seen in the levels of expression of the peptide elongation factor [29]. In skeletal muscle, the embryonic form of this molecule (eEF1A-1) is replaced by an isoform, eEf1A-2, which is the only form expressed in adult skeletal and cardiac muscle and brain. After denervation, as well as during muscle regeneration [28], expression of the adult isoform is sharply reduced and the embryonic isoform greatly increases in prominence.

Aging muscle exhibits many of the same patterns of gene expression as does denervated muscle [15, 19, 30]. In addition to many of the myogenic regulatory factors, levels of Id, a myogenic repressor, are elevated both after denervation and during normal aging [1, 3, 4]. In a moderately old rat (~24 months), levels of many of the molecules that characterize young denervated muscle are commonly elevated severalfold over those seen in young adult control muscle, but they are less than those of young denervated muscle. What is most striking during the aging process in the rat is the dramatic rise in the expression of many developmental genes in the weeks before the expected times of death. These changes are accompanied by increased satellite cell activation and neomyogenesis and would fit the pattern of gene expression that accompanies myogenesis. However, new muscle fiber formation does not account for all of the molecular activity, because substantial myogenin and MyoD expression is also associated with large numbers of myonuclei [21]. The entire picture of molecular and cellular changes is certainly partially associated with the nerve withdrawal seen in old age, but it also appears to reflect what we have termed “homeostatic decompensation” or the breaking down of many of the developmental control mechanisms that normally characterize stable adult muscle. How much this phenomenon applies to other systems of the body remains to be determined.

Conclusions

There are many parallels between the responses of skeletal muscle to denervation and the normal aging process. As an individual ages, the increasing amounts of motor axon withdrawal from a muscle are reflected in structural, functional and molecular changes that are characteristic of denervated muscle. In both long-term denervation and aging, the tissue response is much more complex than might be expected, with atrophic and degenerative processes occurring simultaneously and in the same areas as satellite cell activation, new muscle formation and embryonic patterns of gene expression. What remains to be determined during the aging process is how many of the changes can be attributable to local denervation of muscle fibers and how many occur within the aging muscle fibers themselves.

Acknowledgements

Original research reviewed here was supported by NIH grant PO1 AG 10821.

Address correspondence to:

Bruce M. Carlson, Institute of Gerontology, University of Michigan, 300 North Ingalls Building, #913, Ann Arbor, Michigan 48109 USA, fax 734-936-2116, Email brcarl@umich.edu.

References

[1] Adams L, Carlson BM, Henderson L, Goldman D: Adaptation of nicotinic acetylcholine receptor, myogenin, and MRF4 gene expression to long-term muscle denervation. *J Cell Biol* 1995; 131:1341-1349.

[2] Allbrook DB, Han MF, Hellmuth AE: Population of muscle satellite cells in relation to age and mitotic activity. *Pathology* 1971; 3:233-243.

[3] Alway SE, Degens H, Krishnamurthy G, Smith CA: Potential role for Id myogenic repressors in apoptosis and attenuation of hypertrophy in muscles of aged rats. *Am J Physiol Cell Physiol* 2002; 283:C66-C76.

[4] Alway SE, Degens H, Loew DA, Krishnamurthy G: Increased myogenic repressor Id mRNA and protein levels in hindlimb muscles of aged rats. *Am J Physiol Regul Integr Comp Physiol* 2002; 282:R411-R422.

[5] Ansved T, Larsson L: Effects of denervation on enzyme-histochemical and morphometrical properties of the rat soleus muscle in relation to age. *Acta Physiol Scand* 1990; 139:297-304.

[6] Booth FW, Weeden SH, Tseng BS: Effect of aging on human skeletal muscle and motor function. *Med Sci Sports Exerc* 1994; 26:556-560.

[7] Borisov AB, Carlson BM: Cell death in denervated skeletal muscle is distinct from classical apoptosis. *Anat Rec* 2000; 258:305-318.

[8] Borisov AB, Dedkov EI, Carlson BM: Interrelations of myogenic response, progressive atrophy of muscle fibers, and cell death in denervated skeletal muscle. *Anat Rec* 2001; 264:203-218.

[9] Borisov AB, Huang S-K, Carlson BM: Remodeling of the vascular bed and progressive loss of capillaries in denervated skeletal muscle. *Anat Rec* 2000; 258:292-304.

[10] Brooks SV, Faulkner JA: Contractile properties of skeletal muscles from young, adult and aged mice. *J Physiol (Lond)* 1988; 404:71-82.

[11] Brooks SV, Faulkner JA: Skeletal muscle weakness in old age: underlying mechanisms. *Med Sci Sports Exerc* 1994; 26:432-439.

[12] Brown M, Hasser EM: Complexity of age-related changes in skeletal muscle. *J Gerontol Biol Sci* 1996; 51A:B117-B123.

[13] Carlson BM, Billington L, Faulkner JA: Studies on the regenerative recovery of long-term denervated muscle in rats. *Restor Neurol Neurosci* 1996; 10:77-84.

[14] Carlson BM, Borisov AB, Dedkov EI, Dow D, Kostrominova: The biology and restorative capacity of long-term denervated skeletal muscle. *Basic Appl Myol* 2002; 12:247-254.

[15] Carlson BM, Borisov AB, Dedkov EI, Khalyfa A, Kostrominova TY, Mac Pherson PCD, Wang E, Faulkner JA: Effects of long-term denervation on skeletal muscle regeneration in old rats. *J Gerontol Biol Sci* 2002; 57A:B366-B374.

[16] Carlson BM, Dedkov EI, Borisov AB, Faulkner JA: Skeletal muscle regeneration in very old rats. *J Gerontol Biol Sci* 2001; 56A:B224-B233.

[17] Cederna PS, van der Meulen JH, Faulkner JA, Kuzon WM: Force deficits in aging skeletal muscle: motor unit properties. *Surg Forum* 1999; 50:608-610.

[18] Dedkov EI, Borisov AB, Carlson BM: Dynamics of post-denervation atrophy of young and old skeletal muscles: differential responses of fiber types and muscle types. *J Gerontol Biol Sci*, 2003; 58A: 984-991.

[19] Dedkov EI, Borisov AB, Wernig A, Carlson BM: Aging of skeletal muscle does not affect the response of satellite cells to denervation. *J Histochem Cytochem* 2003; 51:853-863.

[20] Dedkov EI, Kostrominova TY, Borisov AB, Carlson BM: Reparative myogenesis in long-term denervated skeletal muscles of adult rats results in a reduction of the satellite cell population. *Anat Rec* 2001; 263:139-154.

[21] Dedkov EI, Kostrominova TY, Borisov AB, Carlson BM: MyoD and myogenin protein

expression in skeletal muscles of senile rats. *Cell Tiss Res* 2003; 311:401-416.

[22] Gibson MC, Schultz E: Age-related differences in absolute numbers of skeletal muscle satellite cells. *Muscle & Nerve* 1983; 6:574-580.

[23] Gutmann E, Hanzlíková V: *Age Changes in the Neuromuscular System*. Bristol, England, Scientechnica, 1972, 195 pp.

[24] Hashizume K, Kanda K, Burke RE: Medial gastrocnemius motor nucleus in the rat: age-related changes in the number and size of motoneurons. *J Comp Neurol* 1988; 269:425-430.

[25] Ishihara A, Araki H: Effects of age on the number and histochemical [properties of muscle fibers and motoneurons in the rat extensor digitorum longus muscle. *Mech Age Devel* 1988; 45:213-221.

[26] Kadherisan VA, Hassett CA, Faulkner JA: Properties of single motor units in medial gastrocnemius muscles of adult and old rats. *J Physiol (Lond)* 1996; 493:543-552.

[27] Kamińska AM, Fidziańska A, Schulze G, Coper H, Ossowska K, Wolfarth S, Hausmanowa-Petrusewicz I: Ultrastructural changes in the skeletal muscle of senile rats with significant age-dependent motor deficits. *Basic Appl Myol* 1998; 8:185-190.

[28] Khalyfa A, Carlson BM, Carlson JA, Wang E: Toxin injury-dependent switched expression between EF-1α and its sister, S1, in rat skeletal muscle. *Devel Dyn* 1999; 216:267-273.

[29] Khalyfa A, Carlson BM, Dedkov EI, Wang E: Changes in protein levels of elongation factors eEF1A-1 and eEf1A-2/S1, in long-term denervated muscle. *Restor Neurol Neurosci* 2003; 21:47-53.

[30] Kostrominova TY, MacPherson PCD, Carlson BM, Goldman D: Regulation of myogenin protein expression in denervated muscles from young and old rats. *Am J Physiol Regul Integr Comp Physiol* 2000; 279:R179-R188.

[31] Larsson L, Ansved T: Effects of aging on the motor unit. *Progr Neurobiol* 1995; 45:397-458.

[32] Lexell J: Human aging, muscle mass, and fiber type composition. *J Gerontol Ser A* 1995; 50A(Special issue):11-16.

[33] Lexell J, Downham D: What is the effect of ageing on type 2 muscle fibers? *J Neurol Sci* 1992; 107:250-251.

[34] Lu D-X, Huang S-K, Carlson BM: Electron microscopic study of long-term denervated rat skeletal muscle. *Anat Rec* 1997; 248:355-365.

[35] Morley JE, Baumgartner RN, Roubenoff R, Mayer J, Nair KS: Sarcopenia. *J Lab Clin Med* 2001; 137:231-243.

[36] Novotová M, Soukup T: Neomyogenesis in neonatally de-efferented and postnatally denervated rat muscle spindles. *Acta Neuropathol* 1995; 89:85-95.

[37] Pettigrew FP, Gardiner PF: Changes in rat plantaris motor unit profiles with advanced age. *Mech Ageing Devel* 1987; 40:2443-259.

[38] Renault V, Thorne L-E, Eriksson P-O, Butler-Browne G, Mouly V: Regenerative potential of human skeletal muscle during aging. Aging Cell 2002; 1:132-139.

[39] Rodrigues AdC, Schmalbruch H: Satellite cells and myonuclei in long-term denervated rat muscles. *Anat Rec*. 1995; 243:430-437.

[40] Sandri M, Carraro U: Apoptosis of skeletal muscles during development and disease. *Int J Biochem Cell Biol* 1999; 31:1373-1390.

[41] Schmalbruch H, Lewis DM: A comparison of the morphology of denervated with aneurally regenerated soleus muscle of the rat. *J Muscle Res Cell Motil* 15:256-266.

[42] Schröder JM: The fine structure of de- and reinnervated muscle spindles I. The increase, atrophy and hypertrophy of intrafusal fibers. *Acta Neuropath* 1974; 30:109-128.

[43] Viguie CA, Lu D-X, Huang S-K, 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.

[44] Voytik SL, Przyborski MJ, Badylak SF, Konieczny SF: Differential expression of muscle regulatory factor genes in normal and denervated adult rat hindlimb muscles. *Devel Dyn* 198:214-224.

[45] Walters EH, Stickland NC, Loughna PT: The expression of the myogenic regulatory factors in denervated and normal muscles of different phenotypes. *J Muscle Res Cell Motil* 2000; 21:647-653.

[46] Weis J: Jun, Fos, MyoD1, and Myogenin proteins are increased in skeletal muscle fiber nuclei after denervation. *Acta Neuropathol* 1994; 87:63-70.

[47] Zelená J, Soukup T: Increase in the number of intrafusal muscle fibres in rat muscles after neonatal motor denervation. *Neuroscience* 1993; 52:207-218.