Effects of Aging on Regulation of Muscle Contraction at the Motor Unit, Muscle Cell, and Molecular Levels

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Falls are a major cause of morbidity and mortality in the growing population of elderly citizens, and the prevention of falls and gait instability is therefore a very important socioeconomic concern. The mechanisms underlying the increased risk for falls in the elderly are complex and involve aging-related changes in both the central and peripheral nervous system, and in the muscle tissue itself. Further, it has been postulated that impaired neuromuscular function in old age is one of the most important underlying causes of bone fractures in the elderly. In recent years, the strong negative influence of the aging-related loss of muscle mass, strength, and quality (i.e., sarcopenia) on impaired motor function in old age has become increasingly evident. However, our knowledge of the cellular and molecular consequences of the aging process is still incomplete. Therefore, our long-term objective is to have a detailed understanding of the mechanisms underlying the deficits in neuromuscular function present in the elderly.

In an attempt to improve our understanding of the mechanisms underlying the impaired muscle function in old age, we have used different experimental animal models to study aging-related changes in the spatial organization of different motor unit types and regulation of muscle contraction at the motor unit, muscle cell, and molecular levels. This short review will primarily focus on results obtained in our research group during the past 15 years.

The Motor Unit Level

The concept of a motor unit—that is, the single alpha motoneurone and the group of muscle fibers that it innervates and activates—was first introduced by Liddell and Sherrington as the basic unit of motor activity (67). Eccles and Sherrington (24) later emphasized the importance of the motor unit as being the final functional unit in the motor system that forms the basis of all graded muscle contractions. However, it was not until the introduction of the glycogen depletion technique that the spatial organization of the motor unit could be resolved. This technique was originally

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proposed by Krnjevic and Miledi (49) and successfully used first by Edström and Kugelberg (26), Burke et al. (18), Brandstater and Lambert (11, 12), and Doyle and Mayer (23). It was clearly demonstrated that the muscle fibers of a motor unit (motor unit fibers) were scattered in the muscle, resulting in a considerable overlap in the territories of different motor units. In the original studies using the glycogen depletion technique (18, 26), visual evaluation of the staining intensities of various enzymes indicated metabolic homogeneity and a random distribution of motor unit fibers within single motor units. However, visual evaluation only gives a rough estimate of the enzyme activity level and motor unit fiber arrangement. More recent studies using physiological, quantitative enzyme-histochemical techniques and computer-assisted analyses of motor unit fiber distributions have shown a wider range of biochemical and morphological properties than originally proposed, although these variations are considerably smaller than the variation between different units (45, 53).

Spatial Organization of Motor Unit Fibers and Motor Unit Transitions

During the aging process, there is a spatial rearrangement of motor unit fibers and an increased number of muscle fibers per motor unit. At this same time, the total muscle fiber number typically decreases, thus indicating a denervation-reinnervation process (2, 27, 54, 55, 61). This could be secondary to an aging-related degeneration of the subneural apparatus, alternatively a loss of α-motoneurones (for refs., see 54). The altered fast and slow axonal transport and the decreased number of myelinated neurons in ventral roots and peripheral nerves in old age strongly support a fallout of whole motor units as being an important factor underlying the aging-related rearrangement of motor unit fibers and loss of total muscle fiber number (see 54).

Parallel to the spatial reorganization of motor unit fibers, muscle-specific changes in myofibrillar protein isoform expression were observed in both slow- and fast-twitch skeletal muscles in the rat. In the slow-twitch soleus, there is gradual disappearance of the fast-twitch (type II) muscle fibers with age, but the dominating part of this fiber-type shift takes place during growth and maturation (3, 51). This fast-to-slow motor unit transformation has been suggested to be a response to altered functional demands and motoneuron properties related to a gradual increase in body weight (3, 50). Further, the almost complete disappearance of type II fibers, at the age of 12 months, precedes the aging-related loss of total muscle fiber number observed in rats 16 months and older (3). In the fast-twitch tibial anterior and extensor digitorum longus muscle, muscle fiber and motor unit transformations are observed throughout the aging process—that is, it is not a direct consequence of an altered mechanical load related to an increased body weight.

A type IIB to IIX transformation has been reported during the aging process repeatedly by using immunocytochemical (61), immunoblotting (60), and electrophoretic methods (63, 64, 84). In the old animals, the IIX-MyHC units were the dominating motor unit types among the isolated units, whereas in young animals they only constituted a small proportion (59, 61). The reorganization of motor units in old age and the appearance of type IIX MyHC motor unit fibers in regions, which in the young animals are restricted to type IIB motor units, indicate an aging-related motor unit transformation process (Figure 1). Thus, the aging-related decrease of specific motor unit types cannot be exclusively explained by a selective loss of a specific motor unit or muscle fiber type in old age. Finally, a further fast-to-slow transformation
process resulting in an increased number of type IIA and type I fibers has been reported in old age. However, this transformation process appears to be confined to an advanced age (7, 9, 19, 42, 43, 47, 77). Accordingly, it is suggested that the increased number of the IIX-MyHC units in old age reflects an aging-related motor unit transition from type IIB- to IIX-MyHC, possibly preceding a transformation to type IIA-MyHC and following the sequence IIB \( \rightarrow \) IIXB \( \rightarrow \) IIX \( \rightarrow \) IIXA \( \rightarrow \) IIA \( \rightarrow \) I (33, 59).

In young animals, the IIX units have motor unit territories, succinate dehydrogenase activities, and fatigue resistance, which are intermediate between those of the IIA and IIB motor units (59). In the old animals, on the other hand, the biochemical, physiological, and morphological properties of the IIX units resembled those of the IIB units, and the majority of the IIX units were so-called “hybrid units”—that is, they contained muscle fibers which also expressed IIB and occasionally IIA (59, 61). The mechanism underlying the low activity of the mitochondrial enzyme succinate dehydrogenase in the IIX units in old age is not known, but it may be related to (a) a different sequential order of changes during muscle fiber transformation induced by aging as compared with experimentally induced fiber-type transformation, where a change in mitochondrial enzyme activities is one of the first steps in the sequential order of changes where MyHC transitions appear to be one of the final steps; or (b) an impaired adaptability of mitochondrial function in old age. Experimental evidence of morphological (2, 8, 70, 76), as well as functional (68) mitochondrial impairments in old age, supports the latter.

Figure 1 — Camera lucida tracings of the glycogen-depleted fast-twitch motor unit fibers from young animals, classified according to their MyHC composition, in 10 cross-sections of the tibialis anterior muscle from young animals. The superficial part of the muscle is facing the top of the figure. A. Young animals. Motor units expressing type IIA \((n = 3)\), IIX \((n = 6)\), and IIB \((n = 12)\). MyHC isoforms were identified in the young animals. B. Old animals. Motor units expressing type IIA \((n = 1)\), IIA and IIX \((n = 1)\), IIX \((n = 3)\), IIX and IIB \((n = 8)\), and IIB \((n = 3)\). MyHC isoforms were identified in the old animals. The horizontal bar represents 1 mm. From Larsson et al. (61) and Larsson and Ansved (54).
**Contractile Properties**

An increased duration of the isometric twitch has been reported in both fast- and slow-twitch muscles in old age (see 3, 19, 35, 55). The parallel aging-related slowing of the isometric twitch in motor units of both fast- and slow-twitch types indicate that the slowing at the whole-muscle level cannot only be explained by altered motor unit proportions (27, 58, 59, 63). The sarcoplasmic reticulum (SR) properties are the strongest determinants of the speed of contraction of the isometric twitch, whereas the myosin heavy chain (MyHC) isoforms have a major influence on the maximum shortening velocity and the maximum rate of increase in tetanus force (e.g., 13, 37, 52). Thus, an aging-related change in the properties of the SR appears to be a probable explanation for the reduced speed of contraction in old age. The structural (20), functional (58), and biochemical (89) aging-related changes reported in the SR support this idea. Although SR and myofibrillar protein isoforms are expressed in a coordinated fashion, this close coordination is not invariable (92), and may be lost under certain experimental conditions (13, 29). Salviati and co-workers (83) noted that coordinated expression of myofibrillar and SR proteins was lost in skeletal muscle from elderly women. In young rats, a close match was observed between force (twitch and tetanus) and rate of tetanus rise time (reflecting myofibrillar protein function), and the contraction time of the isometric twitch in fast-twitch motor units (reflecting SR properties) was observed in young rats. In the old rats, on the other hand, these coordinated relationships were not observed, indicating an altered coordination of myofibrillar and SR protein function in old age (Figure 2).

![Figure 2](image_url)  
**Figure 2** — Relationship between twitch force, tetanus force, tetanus rise time, and contraction time of the isometric twitch in fast-twitch tibial anterior muscle single motor units from young (3–6 months, filled symbols) and old (20–24 months, open symbols) male rats.
A progressive decline of the maximum contractile force in skeletal muscle is a prominent feature of old age in most mammals. The most important factors in determining the maximum force at the motor unit level are: (a) the number of muscle fibers within the unit (motor unit fibers), (b) cross-sectional motor unit fiber areas, and (c) the capacity of the contractile material to generate maximum force—that is, maximum force normalized to the contractile area of the motor unit fibers (specific tension). The mechanisms underlying the diminished muscle force in old age are controversial and have been discussed in a number of papers (see 15). The major concern has been whether the decline in force is related solely to a loss of contractile material associated with fiber atrophy and/or fiber loss, or whether there is also a decrease in the ability of remaining muscle tissue to generate tension (i.e., an aging-related decrease in specific tension). At the whole muscle level, an aging-related decrease in rodent skeletal muscle-specific tension has been documented in numerous studies (e.g., 4, 14, 21, 46, 78). In these studies, tetanus tensions were normalized to muscle weight (directly or by assuming a constant relation between fiber length and muscle wet mass irrespective of animal age) or to the area of the whole muscle cross-section. However, these estimates of the cross-sectional area of the contractile material do not take into account the gradual replacement of contractile proteins by connective tissue reported in old age (1, 32, 38, 48, 74). Cross-sectional muscle areas and muscle weights will accordingly overestimate the amount of contractile material in old age (3, 55, 63). On the other hand, no significant changes in specific tension were observed at the single motor unit level in rats between the ages of 2 and 24 months, either in fast- or slow-twitch muscles, when tetanus force was normalized to the total motor unit fiber area (3, 27, 28, 30, 55, 59, 60, 72). However, aging-related changes in muscle fiber orientation and glycogen-depletion of motor unit fibers may affect the accuracy of specific tension measurements at the motor unit level. Therefore, aging-related changes in force-generation capacity need to be explored at the muscle fiber or motor protein levels to resolve whether specific tension is affected by aging—that is, if the force loss during aging is also related to a decrease in the number of cross-bridges in the driving stroke or is related to a decrease in the force developed by each cross-bridge.

The Muscle Cell Level

The skinned single muscle fiber preparation has been used in an attempt to improve our understanding of the mechanisms underlying the aging-related changes in contractile speed and force generation. In skinned muscle fibers, membrane structures are rendered permeable, and calcium concentrations can be controlled externally. The structural integrity of the myofilament lattice is maintained, and relevant parameters of contractility, such as force and shortening velocity, can be measured and related to the myofibrillar protein isoforms expressed in the muscle fiber segment. The maximum velocity of unloading shortening (V₀) is probably the most important design parameter of skeletal muscle, since muscles develop their maximum power at a shortening velocity of approximately one-third V₀ (Rome et al. 1990). Thus, to generate power optimally over a wide range of movements, it is crucial to be able to recruit muscle fibers with a wide V₀ range. Myosin is the molecular motor protein that generates force and movement in skeletal muscle. It is a hexamer comprising two MyHC sub-units of approximately 220 kDa mol. wt. and four myosin light chain (MyLC) sub-units of approximately 20 kDa each. In the rat, four MyHC
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isoforms are expressed in trunk and limb muscles from normal adult animals (i.e., the bslow or type I), and the three different fast IIA, IIX, and IIB MyHCs (see Schiaffino & Reggiani 1996). There is a close relationship between V0, the actin-activated ATPase activity of myosin, and the myosin isoform expression of single muscle fibers in different species (e.g., 6, 57, 81). That is, slow fibers express the bslow (type I) myosin heavy chain (MyHC) with a low ATPase activity, whereas fast fibers express fast MyHCs with high ATPase activities. There is good evidence that different forms of myosin confer distinct contractile properties upon muscle fibers. In turn, the contractile properties of a whole muscle result from the aggregate properties and myosin isoform content of its constituent fibers.

**Contractile Properties**

To date, studies on aging-related changes in specific tension at the single fiber level are sparse. Studies have reported an aging-related decline in specific tension varying between 9 and 47% (31, 62, 66, 88), or no decrease in specific tension (25). The reason for the different results is not known, but there is a significant difference in the number of muscle fibers investigated among these studies. In the studies showing an aging-related decline in specific tension, the number of fibers measured varied between 124 and 782. In the study showing no decline in specific tension, only a total of 31 fibers were measured. There is a considerable variability in specific tension between individual muscle fibers, and the precision of specific tension measurements is lower than in, for example, V0 measurements (62). An aging-related change in specific tension may accordingly go undetected if the number of measured fibers is below a critical level.

As discussed above, an aging-related decrease in specific tension could be due to (a) a decrease in the number of cross-bridges in the driving stroke per muscle fiber volume, (b) a decrease in the force developed by each cross-bridge, or (c) a combination of both mechanisms. By using confocal microscopy for high precision muscle fiber volume measurements, aggressive extraction procedures to ensure maximum and consistent protein extraction, and very sensitive electrophoretic methods for protein separation and quantification, recent results from our group have shown a significant aging-related (~12%) decrease in the amount of myosin per muscle fiber volume in old age (71). These data strongly support the hypothesis that the etiology of the aging-related decrease in specific tension is, at least in part, related to a decreased amount of force generating proteins per muscle fiber volume. This is supported by ultrastructural evidence of a myofibrillar loss and of an increase in intermyofibrillar spaces in muscle fibers from 27-month-old rats (2).

An aging-related decline in the maximum velocity of unloaded shortening (V0) was observed in single rat muscle cells, but the slowing was restricted to muscle cells expressing the type I MyHC isoform (Figure 3; 66). The expression of essential myosin light chains (MyLCs) has been shown to modulate V0 in single muscle fibers expressing fast MyHCs in both rats and rabbits (10, 34, 69, 86). In young rats, we have confirmed the modulatory influence of the essential MyLCs on V0 and shown a coordinated expression of MyLC and MyHC isoforms in muscle fibers expressing both type IIX and IIB MyHCs (66). In old age, on the other hand, the modulatory impact of essential MyLCs on V0 and the coordinated expression of MyLCs and MyHCs was partly or completely lost (Figures 4 and 5; 66). The impact of this lack of coordinated expression of MyHCs and MyLCs in old age on regulation of muscle
Figure 3 — Distributions of values for maximum speed of unloaded shortening ($V_o$) from soleus and extensor digitorum longus (EDL) muscle fibers. All soleus fibers expressed only the type I MyHC isoform, while type IIA, IIX, IIXB, and IIB fibers were observed among the EDL fibers in both young (A, B) and old (C, D) animals. From Li and Larsson (66).

Figure 4—Relation between maximum speed of unloaded shortening ($V_o$) and MyLC/MyLC2f ratio in type IIA (filled square), type IIX (filled triangles), type IIXB (open circles), and type IIB (filled circles) extensor digitorum longus fibers from young (A) and old (B) animals. From Li and Larsson (66).
Figure 5 — Interrelationships between maximum speed of unloaded shortening ($V_0$) and the relative contents of MyLC$_3$ and IIB MyHC in type IIXB extensor digitorum longus fibers from young (A, filled symbols) and old (B, open symbols) animals. From Li and Larsson (66).

contraction and the mechanisms underlying this phenomenon remain unknown. The aging-related slowing of $V_0$ was primarily observed in muscle cells expressing the type I MyHC isoform—that is, in a muscle fiber type where MyLC isoform expressions were not significantly affected by aging. It is therefore suggested that the aging-related slowing in type I muscle fibers is secondary to (a) specific changes in the motor protein myosin, such as altered expression of additional slow isoforms, which go undetected with the electrophoretic techniques used or a post-translational modification of the $b$/slow (type I) myosin; or (b) alterations of other myofibrillar or structural proteins in the muscle cell. In an attempt to resolve this issue, we have developed a novel in vitro motility assay that allows studies of myosin function after myosin extraction from single muscle fiber segments (see below).

**Gender-Related Differences and the Response to Hyperthyroidism**

Independent of gender, a similar aging-related fast-to-slow myosin isoform transition has been reported in rodent fast- and slow-twitch hindlimb muscles (22, 65, 94). The phenotypic expression of MyHC isoforms is controlled by developmental, neuronal, hormonal, and mechanical factors, with thyroid hormones being among the most potent regulators of MyHC isoform expression. The aging-related changes in enzyme-histochemical properties and myosin isoform composition in fast- and slow-twitch muscles were reversed by thyroid hormone treatment in both genders. In the soleus, thyroid hormone treatment induced a significant increase in the proportion of type IC and IIC fibers and fast type IIA MyHC and IIX MyHC contents, and a significant decrease in the proportion of type I fibers and type I MyHC contents in both genders (65). However, significant gender-related differences were observed in the response to thyroid hormone treatment (65, 94). In males, all fibers expressed a combination of fast (type IIA and/or IIX) and slow (type
MyHCs in varying combinations and proportions, but there were no pure type I fibers after 4 or 8 weeks of thyroid hormone treatment irrespective of age (66). In the female rats, on the other hand, a more variable expression of MyHC isoform composition was observed, such as type I, type I/IIA, type IIAX fibers, type I/IIAX fibers, cardiac-like fibers, and cardiac-like/IIA MyHC isoforms (66, 94). Finally, in the fast-twitch extensor digitorum longus muscle (EDL), hyperthyroidism induced a decrease in the type IIA MyHC content and increased the type IIB MyHC content in females, irrespective of age. In males, on the other hand, thyroid hormone treatment had no significant impact on the expression of the different type of fast MyHC isoforms in either young or old animals (66). Thyroid, androgen, and estrogen hormone receptors belong to the same nuclear receptor superfamily and have been reported to interact in the regulation of gene expression. It is therefore suggested that the gender-related differences in the MyHC isoform expression in response to hyperthyroidism are related to an interaction between thyroid and sex hormone receptors and a subsequent effect on gene expression (94).

The distribution of $V_0$ values among the single soleus and EDL fibers was similar in male and female rats (22, 94). An aging-related slowing in $V_0$ was observed in single soleus muscle cells, irrespective of gender and thyroid status (22, 94). In hyperthyroid females, the $V_0$ of muscle cells expressing type I MyHC was significantly faster than that in corresponding muscle cells from age-matched controls. This may be related to the fact that only slow MyLC isoforms were expressed in the control soleus, whereas after thyroid hormone treatment, type I fibers expressed both fast and slow MyLC isoforms (94).

The Motor Protein Level

A single muscle fiber in vitro motility assay has been developed in our lab (39, 41) in order to address some of the limitations that exist in conventional motility assays for studying actomyosin function. This novel molecular physiological technique allows detailed studies on specific myosin isoforms that are extracted from a millimeter-long muscle fiber segment directly onto a nitrocellulose coated coverslip. This procedure allows for subsequent analyses of the functional properties of the motor protein. The isolated and immobilized myosin forms a narrow, dense streak on which fluorescent-labeled unregulated actin filaments are propelled in a smooth and bi-directional motion, with highly reproducible motility speed. The native-like motion of sliding actin filaments and the strong correlation between in vitro motility speed and maximum velocity of unloaded shortening as observed in rat and human single fibers expressing various MyHC isoforms (39, 41) offer a unique possibility for comparison of the regulatory influence of different myosin isoforms and thin filament proteins on shortening velocity, at both the molecular and cellular levels.

In Vitro Motility Speed

We have recently employed the single muscle cell in vitro motility assay to improve our understanding of the molecular mechanisms underlying the previously observed aging-related slowing of $V_0$ in rat single muscle fibers expressing the type I MyHC isoform (22, 66, 94). Myosin was extracted from single fibers expressing the slow MyHC and MyLC isoforms of young adult (2–12 months), old (20–24
months), and very old (30 months) rats. An aging-related slowing by 11% and 25% was observed in actin filament sliding speed from the type I myosin of old and very old fibers, respectively, as compared with the myosin isolated from young type I fibers (Figure 6; 39, 41). The result indicates that an aging-related alteration in the mechanical properties of the myosin molecule may be, at least in part, a factor underlying the reported decline in type I single fiber $V_o$. The slowing in motility speed at the molecular level is, however, less dramatic than the ~50% decline in $V_o$ at the cellular level. This difference may be reflected by parallel aging-related changes in structural and regulatory thin filament proteins, which modulate shortening velocity at the single fiber level. The two-fold decrease in in vitro motility speed from myosin of the 30-month-old rats compared with that from the 20–24-month-old rats suggests an accelerated decline in myosin function with advancing age. This observation gains support from a study by Thompson and Brown (1999), in which $V_o$ in rat type I fibers remained unchanged until the age of 24 months, but then decreased by 50% at 30 months of age.

Figure 6 — The distribution of actin filament speeds on type I myosin extracted from single muscle fibers of young (dotted line), 20–24-month-old (solid line), and 30-month-old (dashed line) rats.

Non-Enzymatic Glycosylation (Glycation) of Myosin

Glycation or non-enzymatic glycosylation of proteins has been regarded as one of the biochemical bases underlying the pathophysiology of diabetes and aging (16). Glycation of proteins occurs by a chemical reaction between reducing sugars and the primary amino groups in proteins to form a Schiff’s base linkage. That is, the aldehyde groups of free, unbound sugars react with free amino groups of proteins, forming Schiff’s bases, which undergo further various rearrangements to generate advanced glycation end products, resulting in a slower turn-over of modified proteins and an impaired function of the proteins due to the modification. This is a
common post-translational modification of proteins. To date, most studies on the
effects of aging-related changes of protein structure and function by glycation have
been carried out with extracellular proteins, such as collagen, lens crystallin,
hemoglobin, and albumin.

However, recent studies have shown that intracellular proteins such as
myosin are also affected by the aging-associated glycation process (87, 90). The
reducing sugars primarily target the lysine-rich regions of the proteins, and the two
most lysine-rich regions of the myosin molecule are the actin-binding site and the
ATPase pocket—functionally, the two most important regions of the motor protein.
Decreased actin-activated myosin ATPase activity accordingly has been demon-
strated in response to incubation with glucose and glucose-6-phosphate. However,
the effect of glycation on the mechanical properties of myosin has not been studied
to date. In an attempt to explore the effects of non-reducing sugar exposure on
various myosin isoforms, we incubated myosin extracted from slow- and fast-
twitch fibers with glucose for up to 30 min and measured motility speed in the novel
single muscle fiber in vitro motility assay (80). A significant decrease in actin
sliding speed was observed after 15 min, and motility was reduced to almost zero
after 30 min of incubation.

The turnover rate of skeletal muscle myosin is slow; myosin half-life has been
reported to vary between 14 and 30 days (see 44, 75, 85). A general decrease in
skeletal muscle protein and a specific decrease in myosin turnover rate have been
reported during the aging process (5, 36, 73, 79), making myosin a primary target
for post-translational modifications. A post-translational modification of myosin
by glycation has been reported in old age as well as a negative effect of glycation on
myosin ATPase activity (87, 90). Accordingly, glycation is forwarded as one
potential mechanism underlying the aging-related slowing of contractile speed
observed at the cellular and molecular levels.

Summary

Rodent motor units, muscle fibers, and motor proteins undergo significant aging-
related changes. Such changes include spatial organization and physiological
properties of fast- and slow-twitch single motor units, regulation of contractile
speed and force generation capacity at the muscle fiber level, and altered functional
properties of the motor protein myosin. In addition to specific changes, there also
appears to be a “disorganization” of the coordinated expression of contractile,
sarcoplasmic reticular, and mitochondrial protein isoforms in aging skeletal muscle.
This is suggested to have a strong impact on aging-related impairments in muscle
function in addition to the changes in specific muscle proteins.

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