CURRENT TOPICS FOR TEACHING SKELETAL MUSCLE PHYSIOLOGY

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Contractions of skeletal muscles provide the stability and power for all body movements. Consequently, any impairment in skeletal muscle function results in some degree of instability or immobility. Factors that influence skeletal muscle structure and function are therefore of great interest both scientifically and clinically. Injury, disease, and old age are among the factors that commonly contribute to impairment in skeletal muscle function. The goal of this article is to update current concepts of skeletal muscle physiology. Particular emphasis is placed on mechanisms of injury, repair, and adaptation in skeletal muscle as well as mechanisms underlying the declining skeletal muscle structure and function associated with aging. For additional materials please refer to the "Skeletal Muscle Physiology" presentation located on the American Physiological Society Archive of Teaching Resources Web site (http://www.apsarchive.org).

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A little less than one-half of the body's mass is composed of skeletal muscle, with most muscles linked to bones by tendons through which the forces and movements developed during contractions are transmitted to the skeleton. Contraction is defined as the activation of muscle fibers with a tendency of the fibers to shorten (3). Contraction occurs when an increase in the cytosolic calcium concentration triggers a series of molecular events that includes the binding of calcium to the muscle-regulatory proteins, the interaction of myosin cross-bridges with actin filaments, and the production of the cross-bridge working stroke. Skeletal muscle contractions generate the stability and power for all body movements; consequently, any impairment in skeletal muscle function results in at least some degree of instability or immobility. Muscle function may be impaired as a result of injury, disease, or old age. Impaired muscle function impacts quality of life at all ages, but particularly in the elderly, due to increased risk of severe injury, reduced participation in recreational activities, and ultimately impaired ability to perform activities of daily living and retain one's independence. Thus factors that influence skeletal muscle structure and function are of great interest both scientifically and clinically. The goal of this paper is to update current concepts of skeletal muscle physiology, in particular in relation to effects of injury and aging.

SKELETAL MUSCLE STRUCTURE

Each of the over 600 skeletal muscles in the human body is composed of hundreds to hundreds of thousands of individual, elongated, multinucleated cells called fibers (Fig. 1). Within single fibers, the contractile proteins myosin and actin are incorporated into thick and thin filaments, respectively, which are arrayed in longitudinally repeated banding patterns termed sarcomeres (Fig. 1). Sarcomeres in series form myofibrils, and many parallel myofibrils exist in each fiber. The number of myofibrils arranged in parallel

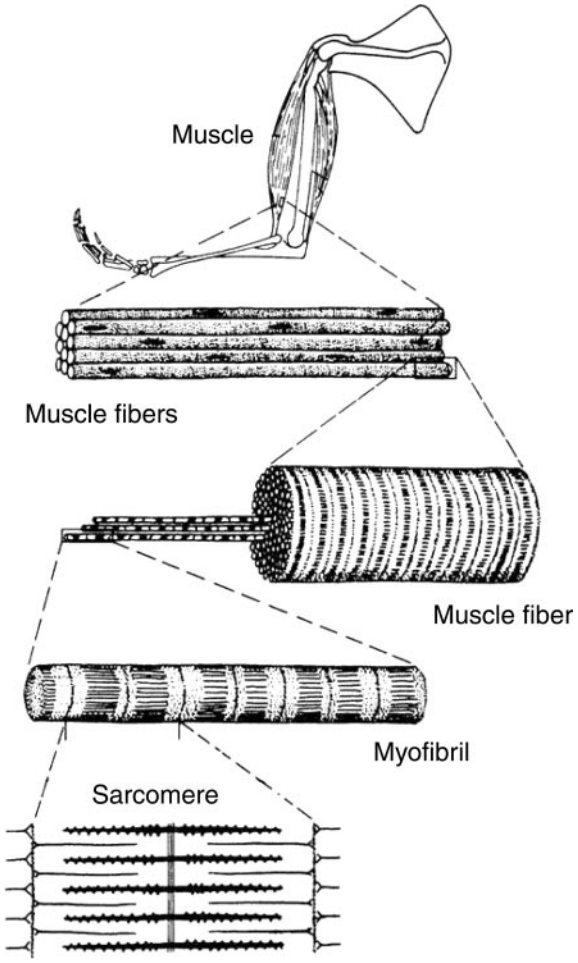


FIG. 1.
Structural hierarchy of skeletal muscle. The hierarchical structure of skeletal muscle is such that muscles are composed of many elongated, parallel-running fibers, with the fibers in turn composed of many approximately cylindrical subcellular structures called myofibrils. The myofibrils display a repeating banding pattern due to the underlying highly ordered array of overlapping myosin thick and actin thin filaments with a single repeat of the banding pattern, referred to as a sarcomere [from McMahon (13a), reprinted by permission of Princeton University Press].

determines the force-generating capability of the fiber. In mammals, the number of fibers in a given muscle is determined at birth and changes little throughout the life span except in cases of injury or disease. In contrast, the number of myofibrils, and, consequently muscle fiber cross-sectional area (CSA), can change dramatically, increasing with normal growth or hypertrophy induced by strength training and decreasing with atrophy associated with immobilization, inactivity, injury, disease, or old age. The change in the length of individual sarcomeres occurs as thick and thin filaments slide past each other, mediated by cyclical interactions between projections from the myosin thick filaments, called cross-bridges, and binding sites on the actin molecules of the thin filament.

Myosin is a molecular motor. Muscle myosin is a hexamer consisting of two so-called heavy chains and two pairs of light chains. The COOH-terminal ends of the heavy chains form a coiled coil of two α-helices that aggregate in the cell to create the backbone of the thick filaments. The remainder of the myosin molecule projects outward from the thick filament, forming the cross-bridge portion of the molecule to which the essential and regulatory light chains are noncovalently bound. Mild proteolytic treatment of thick filaments results in the generation of the light (LMM) and heavy meromyosin (HMM) fragments, and further proteolysis of the HMM cross-bridge fragment gives rise to the subfragments S1 and S2. The myosin S1, also referred to as the "head" of the cross-bridge, retains all of the motor functions of the molecule, i.e., the ability to produce movement and force (15). The crystallographic structure of skeletal muscle S1 shows that the fragment consists of three main functional domains (Fig. 2). The globular NH_2-terminal "catalytic domain" contains both the actin-binding site and the active site for ATP hydrolysis. The long α-helical "neck domain" extends toward the tail of the molecule and appears to be stabilized by its interaction with the two light chains. Finally, between the catalytic and neck domains is the "converter domain" (Fig. 2). A conformational change within S1 while tightly bound to actin appears to provide the "working stroke" that underlies force production and movement (20). Recent advances in structural biology, molecular genetics, and biophysics have come together to enhance significantly our understanding of the nature of the conformational change associated with the cross-bridge working stroke.

The swinging lever arm hypothesis. The current prevailing view is that the cross-bridge working stroke is produced by the angular swinging movement of the neck domain of S1 about a pivot point

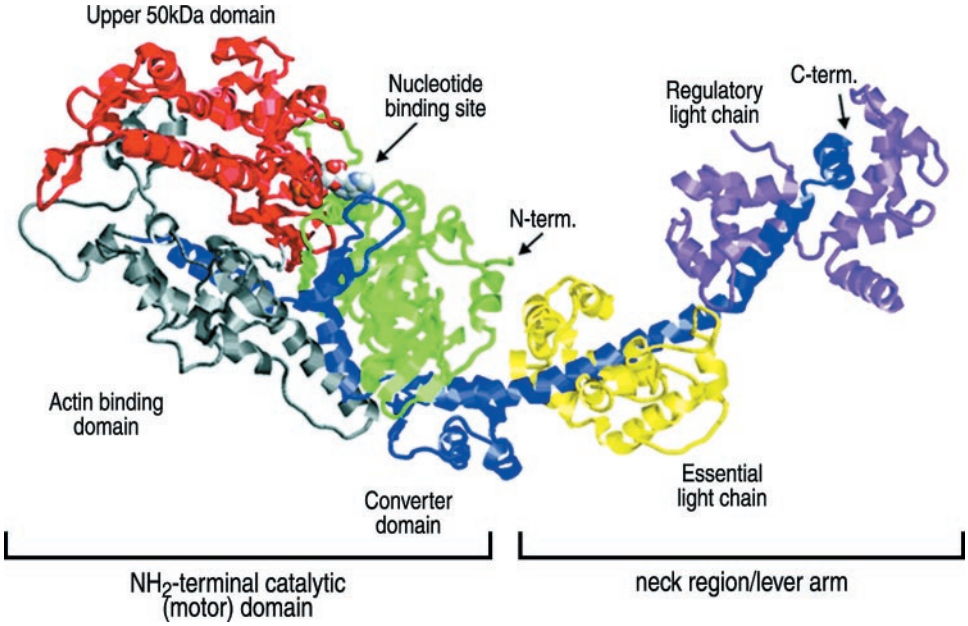


FIG. 2.

Myosin S1 subfragment crystal structure. The ribbon representation is shown for the X-ray crystallographic structure of the myosin head. N-term., NH_2 terminus; C-term., COOH terminus. Green, red, gray, and blue segments constitute the heavy chain; yellow and lavender parts correspond to essential and regulatory light chains, respectively. The fulcrum for the rotation of the neck/lever arm lies within the converter domain. Rotation of the neck domain, with the catalytic domain remaining fairly rigidly attached to the actin filament, appears to provide the basis for the cross-bridge working stroke [modified from Rüegg et al. (16), with permission from the American Physiological Society].

located in the converter domain (Fig. 3). The neck domain of S1 appears to act as a lever arm that "amplifies" small, subnanometer movements associated with the closing and opening of the active site, ultimately producing a displacement at the end of the neck domain of S1 that is on the order of 5–10 nm (7, 20). With the catalytic domain of S1 fairly rigidly attached to actin, the swinging lever arm drives the displacement of the thin filament relative to the thick filament to generate shortening of muscle fibers. Similarly, under circumstances where there is resistance to filament sliding, the same molecular movements result in the deformation of the cross-bridge and the generation of force (15, 20). For an excellent animated representation of the swinging lever arm model of myosin motility, the reader is referred to *Science* online (http://www.sciencemag.org/feature/data/1049155s1.mov).

Strong support for the lever arm hypothesis of the working stroke is provided by ingenious and very elegant experiments assessing the effects on myosin function of altering, through molecular genetic techniques, the length of the lever arm (16, 19). In these studies, both the speed at which myosin translated actin filaments in an in vitro motility assay (19) as well as the magnitude of the displacement of the actin filaments during a single cross-bridge working stroke (16) varied linearly with the length of the "engineered" lever arms. The predictable changes in the function of the molecule extended to improved function, i.e., faster speeds and larger steps compared with control values when the length of the lever arm was increased.

SKELETAL MUSCLE FUNCTION

Chemomechanical coupling. During contraction, individual myosin cross-bridges attach to actin, cycle through the still not fully defined chemical and mechanical states that constitute the working stroke, detach, and then reattach (Fig. 4). This so-called cross-

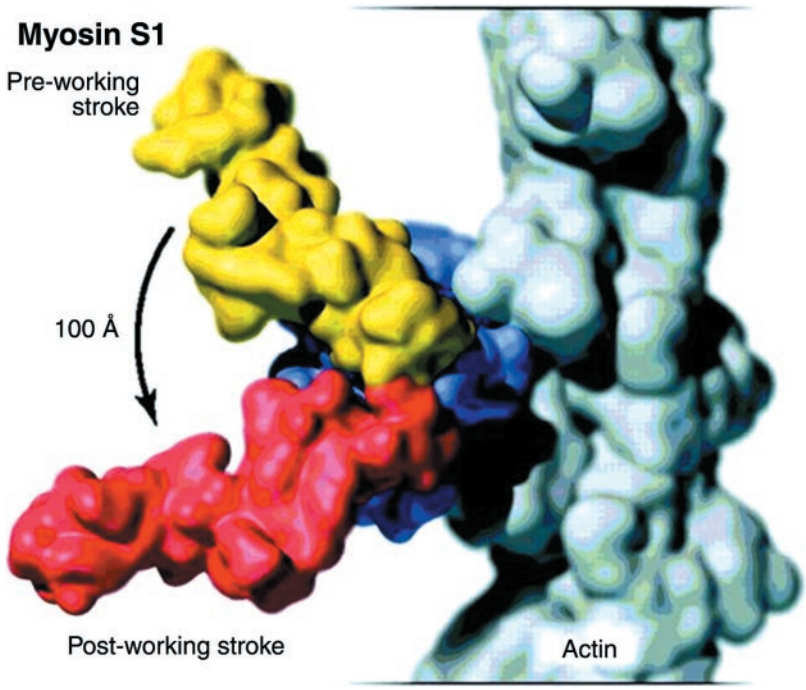


FIG. 3.

Swing lever arm of the myosin working stroke. A model of myosin complexed with an actin filament demonstrates an ~100-Å motion of the neck domain/lever arm generated when the motor undergoes a transition from an ADP · P_i-bound (preworking stroke) state to an ADP/nucleotide-free (postworking stroke) conformation. The figure was generated by superimposing the structures of ADP-A1F_4^- smooth muscle myosin and nucleotide-free chicken skeletal myosin. Shown are converter/neck domain positions in ADP · P_i (yellow) and nucleotide-free (red) states, the similar catalytic cores (blue), and the actin filament (gray) [image reprinted from Vale and Milligan (20) (online http://www.sciencemag.org)]. Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part without prior written permission from the publisher.

bridge cycle is driven in one direction by coupling the transitions between cross-bridge states to the steps of the hydrolysis of adenosine 5′-triphosphate (ATP) by myosin. A single molecule of ATP is hydrolyzed for each working stroke of the myosin cross-bridge. Overall, the cross-bridge cycle depicted in Fig. 4 can be summarized as consisting of the following key transitions: ATP binding to myosin, resulting in the dissociation of actin and myosin; ATP hydrolysis and the subsequent reassociation of actin and myosin; and the release of the hydrolysis products. Two steps in the ATP hydrolysis reaction in muscle, ATP binding to myosin and phosphate release from myosin, are highly favorable energetically and act to drive the cycle from left to right, as illustrated in Fig. 4. Whether the working stroke is coupled directly to the release of phosphate from AM · ADP · P_i (where A is actin and M is the head of the myosin molecule), to an isomerization of AM · ADP · P_i or AM · ADP, to the dissociation of adenosine 5′-diphosphate (ADP) from AM · ADP, or to some combination of these steps is as yet unknown (7). The coupling of the hydrolysis of a

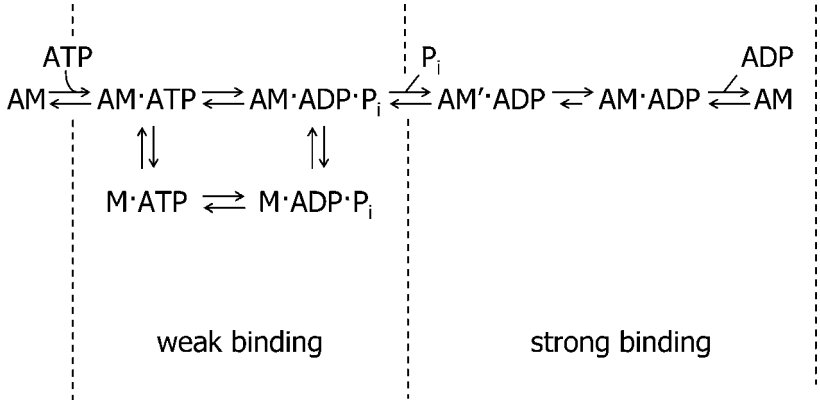


FIG. 4.

Cross-bridge cycle. Schematic model is shown for the actomyosin ATP hydrolysis reaction during contraction of skeletal muscle, where A is actin and M is the head of the myosin molecule. The actin-myosin interactions designated "weak" are not force- or movement-generating states. The transition from "weak" to "strong" actin-myosin interactions is closely associated with the cross-bridge working stroke. The phosphate release step has been hypothesized to be tightly coupled to this transition on the basis of the observation that addition of phosphate during contraction causes a decrease in force generation. In contrast to this apparent reversibility of the phosphate release step, addition of ADP to fibers in rigor, with the rigor state corresponding to the actomyosin state with no nucleotide bound (AM), does not lead to relaxation, and the addition of phosphate to the AM · ADP state also does not relax tension. Consequently, AM′ · ADP and AM · ADP are both included in the scheme, as they are presumed be different chemical states, with the transition from AM′ · ADP to AM · ADP difficult to reverse [redrawn from Goldman and Brenner (8), with permission from the *Annual Review of Physiology*, vol. 49 (1987) by Annual Reviews (www.annualreviews.org)].

single molecule of ATP to each cross-bridge working stroke necessitates that the rate of cross-bridge cycling is determined by the ATPase activity. The rate of cycling establishes how rapidly the thick and thin filaments can slide past one another and therefore how rapidly a muscle fiber can shorten. Thus velocity of shortening is determined by ATPase activity, which in turn is determined primarily by myosin heavy-chain (MHC) isoform.

Muscle myosins. Each myosin heavy-chain isoform has its own characteristic ATPase activity. There are at least seven separate skeletal muscle MHC genes that in human beings are arranged in series on chromosome 17 (9). These include, in addition to the adult isoforms, both an embryonic and a neonatal MHC gene. Two cardiac MHC genes are located in tandem on chromosome 14, with the β-cardiac MHC being the predominant gene expressed in slow skeletal muscle fibers (9). There are several subtypes of fast MHCs. Types 2A and 2X (also known as 2D) are the fast MHC isoforms expressed in the skeletal muscles of human beings, and an additional, very fast myosin, type 2B, is found in the muscles of rodents and other small mammals (9). The β-cardiac MHC is a myosin with a long ATPase cycle time that, as a consequence, uses ATP slowly. The fast myosins have more rapid ATPase cycle times; therefore, fibers expressing these myosins can achieve much higher shortening velocities.

In vivo, muscles may shorten, remain at a fixed length, or be lengthened during contractions. The type of contraction that occurs depends on the interaction between the magnitude of the force developed by the muscle and the external load placed on the muscle (3). When the force developed by the muscle is greater than the load on the muscle, the fibers shorten during the contraction. When the force de-

veloped by the muscle is equal to the load or if the load is immovable, the overall length of the muscle remains the same, resulting in an isometric contraction. If the force developed by the muscle is less than the load placed on the muscle, the muscle is stretched during the contraction. In general, the initiation of a movement or the acceleration of a body part requires a shortening contraction and the generation of power (force $\times$ velocity), whereas braking actions and deceleration result in lengthening contractions. Most activities require varying proportions of each type of contraction, but clearly the ability to generate power, rather than simply isometric force, is the most physiologically relevant marker of performance. With little evidence of significant differences between MHC isoforms in terms of force development, power output is determined by shortening velocity.

A single muscle fiber is innervated by a single branch of a motor neuron, whereas a single motor neuron branches to innervate many muscle fibers. A motor nerve, its branches, and the muscle fibers innervated by the branches constitute a motor unit. The motor unit is the smallest group of fibers within a muscle that can be activated volitionally. Activation of a motor unit occurs when action potentials emanating from the motor cortex depolarize the cell bodies of motor neurons. The depolarization generates an action potential in a motor neuron that is transmitted to each muscle fiber in the motor unit, and each fiber then contracts more or less simultaneously. Motor units range in size from small units with perhaps only several dozen muscle fibers to large units containing many thousands of fibers. Although modulation of force does occur at the level of individual motor units, motor units are most often recruited at a frequency that results in near-maximum activation of the fibers in the motor unit. Consequently, increasing the number of active motor units is the most important physiological means of controlling muscle force generation and power output in vivo.

Physiological profiles of motor units. Motor units are classified functionally on the basis of their mechanical and metabolic properties, namely speed of shortening and resistance to fatigue. Speed of shortening is determined by MHC isoform, as already described. Fatigue resistance is a function of the ability of the muscle fibers to establish and maintain energy balance; that is, energy in the form of ATP must be replenished as rapidly as it is utilized. The ability to generate ATP is governed primarily by the concentration and activities of the enzymes of oxidative metabolism, or the oxidative capacity of the fibers. Skeletal muscle is enormously plastic in that it adapts its oxidative capacity and probably MHC expression to match the habitual level of demand for physical activity placed on it. Level of physical activity is defined in terms of frequency of recruitment and loading. Because all of the muscle fibers in a motor unit contract simultaneously, the pressures for gene expression associated with activity are identical in all of the fibers of the motor unit, and, as a consequence, all of the fibers within a motor unit have the same biochemical and, therefore, functional properties. The details of how the effects of frequency and loading are transduced into fiber type determination are only beginning to be understood at the molecular level (9, 13).

Slow (S) motor units have the smallest single muscle fiber CSAs, the fewest muscle fibers per motor unit, and the lowest velocity of shortening. Slow muscle fibers contain many mitochondria, giving the S motor units a high capacity to replenish ATP. As a consequence of their small size and slow shortening velocity, S units are recruited during tasks that require low force or power but highly precise movements. The low rate of ATP usage also results in the economical maintenance of force during isometric contractions as well as efficient performance of repetitive, slow, shortening contractions. Fast motor units, with their larger sizes and high shortening velocities, are recruited under circumstances when high power output is needed or when isometric force produced by slow motor units is insufficient. The fast motor units are classified further as fast fatiguable (FF) or fast fatigue resistant (FR). FF motor units are composed of the largest muscle fibers, have the most muscle fibers per motor unit, and have the highest velocities of shortening. The FF units are the last to be recruited and are recruited for only individual or very short-duration high-force contractions or high-power movements. The FR units are intermediate in terms of the CSAs of their fibers, the number of fibers per motor unit, the velocity of shortening, and the frequency of recruitment. The force normalized per unit CSA is $\sim$280 kN/m^2 for each type of fiber, but the maximum normalized power (W/kg) developed by FF units is as

much as fourfold greater than that of the S units due to the higher velocity of shortening for the fibers in the FF units.

AGING

Between maturity and old age, a wide variety of species, including human beings, show a 30–40% decrease in muscle mass and even greater decreases in the development of maximum force and power (1). In addition, one-third to one-half of people over age 65 years experience at least one fall per year, and falls contribute significantly to morbidity and mortality. Although a cause-effect relationship between muscle strength and falling has not been established, compared with age-matched control subjects, subjects with a history of frequent falls showed significantly lower values for strength of the muscle groups associated with balance. This and other correlative studies support the role of a loss in muscle strength and power as a major contributor to increased incidence of falling with aging (1). Although the muscle atrophy and weakness associated with aging may be related to decreased levels of physical activity in aging populations, maintenance of activity does not protect skeletal muscles completely from age-related decrements. Even superbly trained world-class athletes show similar rates of decline with aging in skeletal muscle structure and function compared with untrained subjects (1). Furthermore, although record-setting performances have improved by 20–90% over a century of Olympic competition, these performances have consistently occurred in early adulthood (4).

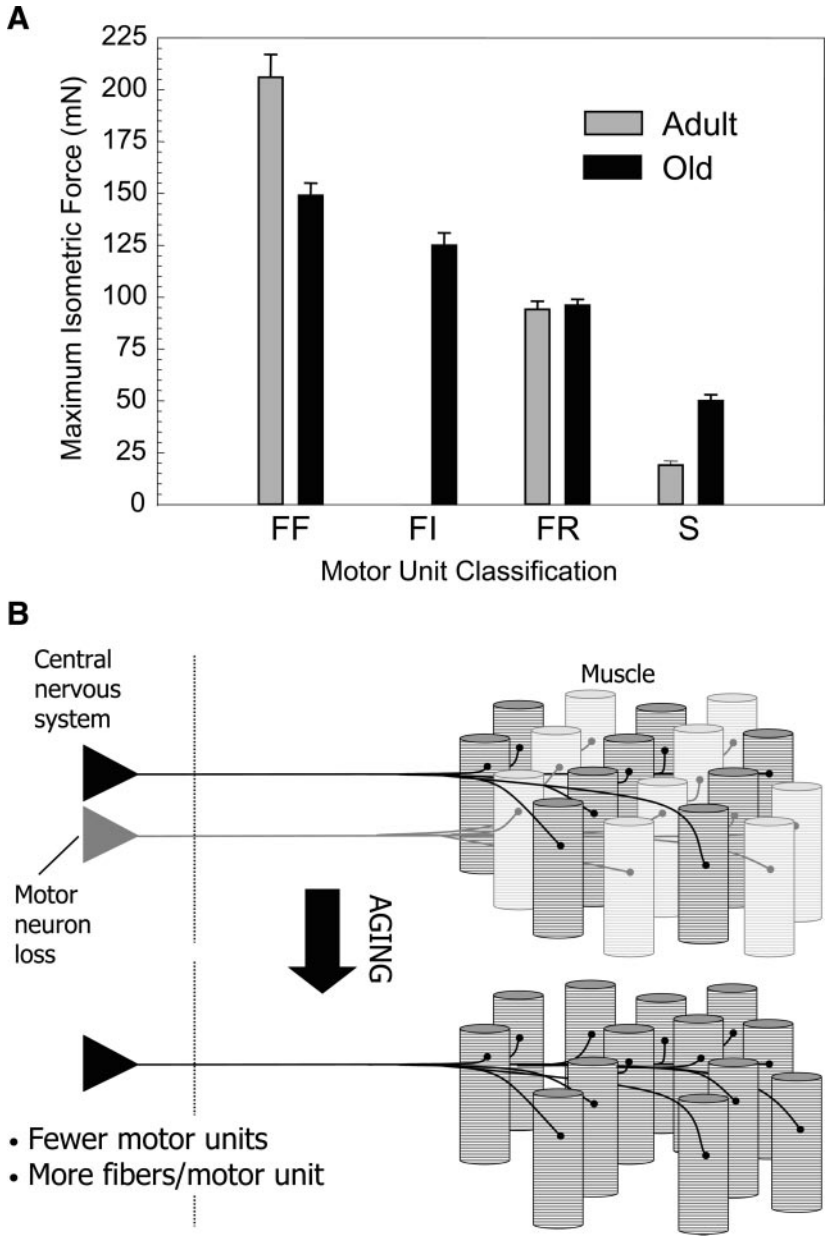
Despite the significance of skeletal muscle atrophy, weakness, and physical frailty as inevitable concomitants of old age, the mechanisms responsible for these impairments are only partially understood (1, 4). Age-associated muscle atrophy is the result of a combination of individual-fiber atrophy, which is potentially reversible or preventable through exercise, along with a decrease in the total number of fibers, which is likely irreversible. Fiber number appears to decrease as a result of the loss of whole motor units. For medial gastrocnemius muscles of rats, the number of FF units decreased in old age by approximately one-third compared with adult values (11). In contrast, the number of S units remained constant with aging, but the number of fibers per motor unit increased threefold in old age (11). In addition, the mean maximum force of FF motor units in old rats was 70% that of comparable motor units in adult rats, whereas that of S motor units was 250% of the adult value (Fig. 5*A*). On the basis of these data, the conclusion is that the age-related remodeling of motor units involves selective denervation of fast muscle fibers with reinnervation of some of the denervated fibers by axonal sprouting from slow fibers (Fig. 5*B*). Presumably, fibers that are not reinnervated undergo denervation atrophy and ultimately disappear entirely. Indirect estimates of motor unit size and number along with direct histological evidence of fiber type grouping indicate a similar process of motor unit remodeling in the muscles of elderly human beings (1). The net result of the loss and atrophy of individual fibers and the loss of fast motor units with increasing size of slow motor units includes muscle atrophy, lower maximum force, still lower maximum power, decreased rate of force generation, and loss of fine motor control. Finally, in addition to the loss in muscle mass and consequent loss in muscle force, muscles of old animals show a deficit of ~20% in maximum specific force normalized for muscle CSA, suggesting qualitative as well as quantitative deficiencies associated with aging (1).

CONTRACTION-INDUCED INJURY

Injury to skeletal muscles may occur as a result of disease, such as dystrophy; exposure to myotoxic agents, such as bupivacaine or lidocaine; sharp or blunt trauma, such as punctures or contusions; ischemia, such as that which occurs with transplantation; exposure to excessively hot or cold temperatures; and most commonly the muscles' own contractions. Regardless of the factors responsible, the manner in which the injuries are manifested appears to be the same, varying only in severity. In addition, the processes of fiber repair and regeneration appear to follow a common pathway regardless of the nature of the injurious event. Contraction-induced muscle injury is most likely to occur during activities that involve predominantly the lengthening of the muscles during contractions. Skeletal muscles intermittently experience contraction-induced injuries throughout the life span. Three critical changes occur in the skeletal muscles of old animals: muscle fibers are injured more easily; muscle fibers regenerate less

well; and, after severe injuries, structural and functional recovery is not complete (4). Consequently, contraction-induced injury may provide a possible mechanism for the gradual development with aging of muscle atrophy and the decreases in specific force and normalized power.

Mechanisms underlying contraction-induced injury. The initiating event associated with contraction-induced injury is primarily mechanical in nature, occurring when individual sarcomeres are stretched excessively, damaging some structural component within or between sarcomeres. The injury may involve any number of fibers within a muscle, and, within an individual fiber, both focal injuries, which are localized to a few sarcomeres in series or in parallel, and more widespread injuries, across the entire cross section of the fiber, are observed with



electron microscopic techniques (4). A promising hypothesis is that injury is initiated when weak sarcomeres are stretched by stronger sarcomeres that exist in series. Thus the greater susceptibility to contraction-induced injury of muscles in old animals would arise from a larger population of weak sarcomeres in muscles fibers of old compared with adult animals. Direct morphological evidence of damage is critical to confirm that an injury has occurred, but because of the focal nature of the injury, its magnitude cannot be quantified by direct methods. Indirect measures of injury include soreness, plasma concentrations of creatine kinase or lactate dehydrogenase, and a decrease in maximum force. The subjectivity of reports of soreness and the variability in serum enzyme levels limit their usefulness as measures of muscle damage. Although immediately after an exercise protocol the decrease in force reflects both fatigue and injury, the deficit remaining following recovery from fatigue in the ability of the muscle to develop force provides the most quantitative and reproducible measure of the totality of a muscle injury.

The initial injury gives rise to a cascade of events that lead, over the course of several days, to a secondary injury, represented by a peak in the force deficit and in the number of fibers in a cross section that demonstrate overt evidence of morphological damage. A major component of the secondary injury involves the infiltration of the damaged muscle by inflammatory cells (18). Phagocytic cells act to remove the disrupted myofilaments, other cytosolic structures, and the damaged sarcolemma. The most severe injuries result in the complete degeneration of the muscle fiber, leaving only the empty basal lamina, which appears to be highly resistant to any type of injury and generally remains intact (2). Because of the role of inflammatory cells in the manifestation of the secondary injury, the use of anti-inflammatory drugs may seem beneficial for limiting the extent of the damage. An important consideration, however, is the role of the infiltrating cells, not only in mediating damage but in the activation of repair processes necessary for successful recovery from the damage (18).

FIG. 5.

Motor unit remodeling. *A*: graph shows mean motor unit forces for fast fatiguable (FF), fast fatigue-resistant (FR), and slow (S) motor units in medial gastrocnemius muscles of adult and old rats. Compared with motor units in adult rats, for muscles of old rats FF motor units generated ~30% lower forces due to a combination of individual muscle fiber atrophy and decreased specific forces, and S motor units generated nearly threefold higher forces due to increased numbers of muscle fibers per motor unit. In addition, a population of motor units, termed fast intermediate (FI), was identified in muscles of old rats, with properties not entirely consistent with the functional classification scheme established for FF, FR, and S motor units [data from Kadhiresan et al. (11)]. *B*: schema of the motor unit remodeling process that occurs in skeletal muscle with aging. Selective loss of fast motor neurons (gray) leaves the fibers of that motor unit denervated. Axonal sprouting from adjacent, typically slow motor neurons (black) reinnervates some of the denervated fibers. The result is fewer, larger motor units. In addition, whereas in young, nondiseased muscle individual fibers from a given motor unit are very infrequently found adjacent to one another, in the muscles of old animals this grouping of several fibers from a single motor unit is frequently observed.

Recovery from contraction-induced injury. Under circumstances when the injury involves only minor disruptions of the thick or thin filaments of single sarcomeres, the damaged molecules are likely replaced by newly synthesized molecules available in the cytoplasmic pool (17). After more severe injuries, regeneration of the damaged section of the fiber or even of the entire muscle fiber will occur.

Satellite cell activation. A key element in the initiation of muscle fiber regeneration following a wide variety of injuries is the activation of satellite cells (2). Satellite cells are quiescent myogenic precursor cells located between the basal lamina and the sarcolemma. Upon activation, satellite cells divide mitotically to give rise to myoblasts. Proliferation of satellite cell-derived myoblasts provides new myonuclei to muscle fibers that are increasing in size during growth or hypertrophy. Alternatively, the myoblasts can fuse with each other to form multinucleated myotubes acting locally to repair an injured section of fiber or generating a completely new fiber within the remaining basal lamina of a degenerated fiber. The myotubes ultimately differentiate completely into adult muscle fibers (2). Satellite cells may be activated by factors endogenous to the injured tissue itself or synthesized and secreted by other cell types at the wound site, including infiltrating neutrophils and macrophages (10). The fibroblast growth factors, platelet-derived

growth factor, transforming growth factor-β, and the insulin-like growth factors are among the factors that activate and regulate satellite cell function, and the effects of these factors on muscle satellite cell proliferation and differentiation have been studied extensively in cell culture (6). In addition to the activation of resident satellite cells, regenerating muscle may also recruit undifferentiated myogenic precursor cells from other sources.

Myogenic regulatory factors. New muscle cell formation from muscle satellite cells resembles embryonic development in the sense that, in regenerating muscle cells, embryonic isoforms of the muscle proteins are expressed. The conversion of satellite cells to mature fibers in both developing muscle and regenerating muscle is directed by a group of related regulatory factors. These so-called muscle-regulatory factors (MRFs) are part of a superfamily of basic helix-loop-helix DNA-binding proteins that interact to regulate the transcription of skeletal muscle genes (14). The MRF family can be divided into two functional groups: MyoD and Myf-5 are referred to as primary factors and are required for the determination of skeletal myoblasts, whereas myogenin and MRF4 are secondary factors that act later and are necessary for differentiation of myoblasts into myotubes. Gene-targeting experiments, in which null mutations were introduced in each of the MRF genes, support separate and distinct roles in myogenesis for each of the MRFs (14).

Adaptation for protection from injury. Anecdotal reports by human beings of prior exposure to an injurious activity providing protection from delayed-onset muscle soreness have been supported by experiments demonstrating decreased amounts of muscle damage with repeated exposures of rats to downhill running. In addition, prior exposure of muscles to a single bout of lengthening contractions reduced the force deficit and the number of damaged fibers following exposure to the same lengthening contraction protocol administered two weeks later (Fig. 6). The mechanisms responsible for the protection are not known. One hypothesis is that training with lengthening contractions may eliminate injury-susceptible fibers, or parts of fibers, leaving muscle fibers that are less susceptible to injury. Consistent with this hypothesis is the observation that degeneration/regeneration, whether due to contraction-induced injury, injection of myotoxin, or disease, is sufficient to provide protection from contraction-induced injury. Despite the effectiveness of degeneration/regeneration for producing protection from contraction-induced injury, conditioning with protocols of either repeated isometric contractions or stretches with the muscle relaxed, neither of which result in any evidence of degeneration/regeneration, also provided protection from lengthening contraction-induced injury two weeks later (Fig. 6). These data indicate that

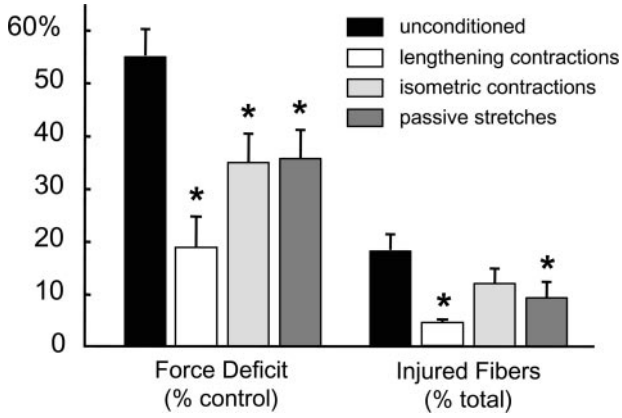


FIG. 6.

Conditioning with single exposures to a protocol of lengthening or isometric contractions or stretches without stimulation. Force deficits and percentages of injured fibers in cross sections of extensor digitorum longus muscles of mice were measured 3 days after muscles were injured by a bout of 75 lengthening contractions administered to groups of mice that were not conditioned (unconditioned) or 2 wk after conditioning with an identical bout of lengthening contractions (lengthening contractions), a bout of 75 isometric contractions (isometric contractions) or 75 stretches without stimulation (passive stretches). Force deficits were calculated as the difference between the maximum isometric forces produced before and 3 days after the injury bout, expressed as a percentage of the preinjury value. Injured fibers were calculated as the number of fibers in a cross section determined to exhibit clear signs of degeneration, expressed as a percentage of the total number of fibers in the section. Values are means ± SE. Force deficits and percentages of injured fibers for all groups were significantly greater than 0 ($P < 0.05$). *Significant difference from the unconditioned group ($P < 0.05$) [modified from Koh and Brooks (12), with permission from the American Physiological Society].

lengthening contractions and fiber degeneration/regeneration are not required to induce protection from lengthening contraction-induced injury. The therapeutic implications of these findings may be especially relevant for elderly populations, for whom training with damaging lengthening contractions may be particularly risky due to the increased susceptibility to injury of muscles in old animals coupled with the decreased capacity for recovery. In addition, a significant fraction of the elderly population may be unable or unwilling to exercise at a sufficient intensity to elicit protective adaptations. Maintenance of conditioned muscle fibers, particularly in muscles of elderly people, may be highly effective for prevention of inadvertent damage during contractions.

THERAPEUTIC INTERVENTIONS

When an injury or impairment is so severe that the total replacement of the muscle is required, a whole donor muscle may be transposed or transplanted into the recipient site (5). Small free standard grafts have long been popular for treatment of patients with partial facial paralysis. Transplantation of large skeletal muscles with immediate restoration of blood flow through the anastomosis of the artery and vein provides an operative technique with numerous applications for reconstructive surgery as well as for treating impairments in function of the limbs, anal and urinary sphincters, and even the heart. Transplantation of muscles invariably results in structural and functional deficits, with tenotomy and repair being the major factors responsible (5). The deficits are of the greatest magnitude during the first month, and then a gradual recovery results in the stabilization of structural and functional variables at levels between 60 and 70% of control by 120 days (5). Despite the deficits, transposed and transplanted muscles develop sufficient force and power to function effectively for maintenance of posture and patent sphincters and to move limbs or drive assist devices in parallel or in series with the heart.

Myoblast transfer and gene therapies hold great promise for individuals afflicted with inherited myopathies such as muscular dystrophy. These approaches are aimed at delivering exogenous genetic constructs to skeletal muscle cells. Myoblast transfer therapy involves intramuscular injection of myoblasts containing a normal, functional genome. The concept of myoblast transfer is based on the role that satellite cells play in muscle fiber growth and repair. The idea is to obtain satellite cells containing a functional gene from a healthy compatible donor, have the cells multiply in culture, and then inject the "normal" myoblasts into the muscles of the patient. The objective is for the injected myoblasts to fuse with growing or regenerating muscle fibers to form a mosaic fiber in which the cytoplasm will contain at least some normal myoblast nuclei. Gene therapy presents a more complex and flexible approach, whereby genetically engineered DNA constructs are delivered to a host cell specifically to direct production of a desired protein. By reengineering the coding sequence of the gene and its regulatory regions, the function, the quantity of expression, and/or the protein itself can be altered. Consequently, the challenges behind gene therapy are not only obtaining a functional construct of a given gene and regulatory region but designing effective delivery techniques of the gene, in the case of skeletal muscle, to an enormous number of cells throughout the entire body. Methods to transfer genetic material into a muscle cell include direct injection and the use of retroviral and adenoviral vectors. Despite their promise, each of these strategies presents highly technical difficulties that to date remain unresolved.

DISCLOSURES

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