Chapter 11: Muscle Physiology

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Some of the most perplexing clinical problems confronting surgeons of the head and neck are closely linked to the physiology and pathophysiology of skeletal muscle. Facial paralysis and laryngeal paralysis, for example, are "double-deficit" disorders with abnormalities that often include both neural and muscular components. Few would argue that our current ability to rehabilitate patients with these disorders is completely satisfactory. Optimal treatment necessitates a working knowledge of the physiology and pathophysiology of both the neural and muscular components. The focus here will be on skeletal muscle.

The purpose of this chapter is to review the basic principles of skeletal muscle physiology, and to highlight the unique aspects of skeletal muscle of the head and neck region. A brief overview of the techniques used by muscle physiologists to evaluate skeletal muscle also will be presented.

Structure of Skeletal Muscle

The gross structure of skeletal muscle in the head and neck region varies widely. The sternomastoid muscle, for example, is a large, well-defined muscle with tendinous origins and insertions from bone. Facial mimetic muscle, in contrast, is wispy and diffuse peripherally, organized into definable muscle bundles more centrally, and has origins and insertions more often in skin and soft tissue than bone. The force vectors of head and neck muscle are often complex. The omohyoid or digastric muscles have fixed central tendons with different force vectors produced by the anterior and posterior bellies. The tendons of the oblique extraocular muscles and the tensor veli palatini pass around a pulley and exert force in a direction completely different from their direction of contraction.

The familiar whole muscles of the head and neck region are composed of thousands of individual *muscle fibers* (Fig. 11-1). The fibers are multinucleate cylinders ranging from 10 to 100 microns in diameter and from a few millimeters to several centimeters in length (Guyton, 1979). The fibers may be arranged parallel to the long axis of the muscle or they may insert obliquely into a central tendon running longitudinally within the muscle. The latter arrangement is termed *pennate* or *bipennate architecture,* reminiscent of the individual fibers of a feather inserting into the feather's central quill (Gans, 1982). The muscle fibers of a skeletal muscle vary structurally and biochemically according to the functional and metabolic demands of that particular muscle.

Similar to nerve fibers, muscle fibers are often grouped within the whole muscle into *fascicles.* The membrane surrounding each muscle fiber is called the *sarcolemma.* The connective tissue layer investing each fascicle of muscle fibers is the *perimysium.*

Each muscle fiber contains up to several thousand *myofibrils.* These are the filamentous aggregates of thousands of myosin and actin molecules.

The smallest functional unit of skeletal muscle is the sarcomere (Fig. 11-2). This is the physiologically defined contractile unit that is contained by adjacent Z-lines (Eisenberg,

1983). The details of the sarcomere are visible on electron microscopy (Fig. 11-3).

The proteins that make up the sarcomere include actin, myosin, troponin, and tropomyosin. As will be discussed in more detail below, the myosin molecule is the more active of the four in the contractile process. The myosin molecule is a multi-subunit protein consisting of two heavy peptide chains (Gershman, Stracher, and Dreizen, 1969; Julian, 1971) and four light peptide chains (Weeds and Lowey, 1971; Fig. 11-4). Variations in the light and heavy chains of myosin result in several isoforms of the molecule that are biochemically and functionally unique (Hoh and Yeoh, 1979). Thus, at the level of the myosin molecule, the functional characteristics of the sarcomere and, ultimately, of the entire muscle fiber, are determined.

The actin molecule is a double-stranded, filamentous, "coiled-coil" protein sequence (Fig. 11-5). Spaced along the actin molecule are the binding sites for the myosin cross bridges, which are activated during contraction. Within the groove of the actin coil lies the filamentous tropomyosin molecule. Spaced intermittently along the tropomyosin molecule are the more globular troponin molecules. Troponin and tropomyosin exist in a 1:1 ratio and together they seem to exert an inhibitory effect on the actin-myosin cross-linking (Guyton, 1979).

At the contractile protein level, skeletal muscle of the head and neck region may prove to be structurally identical to skeletal muscle elsewhere in the body. Some unique and not easily explained features of the skeletal muscle derived from branchial arch mesoderm have been detected, however, such as the extremely fast yet fatigue-resistant contractile properties of intrinsic laryngeal muscles (Hall-Craggs, 1968) and the unexpected fiber type transformation seen in facial mimetic muscle of the rabbit following denervation (Sillman and Faulkner, 1990). Further physiologic analysis of normal and diseased skeletal muscle of the head and neck region should indicate whether these unique features are caused by compositional differences in head and neck muscle or are simply a result of intricate muscle-bundle architecture and complex neural programming.

Fiber types and structure

Most whole skeletal muscles are composed of fibers of varying types. Muscle fibers may differ with respect to size, color, contractile force, velocity, fatigue characteristics, and biochemical composition. Most of these variables have been used at some point to classify muscle fibers into different subtypes. It is difficult to compare and correlate fibers classified by different investigators based on differing variables (Eisenberg, 1983). Engel (1962) introduced perhaps the most widely applied system of fiber classification, which is based on histochemical staining of the fibers for ATPase. This system was further modified by Brooke and Kaiser (1970). In this system, type I fibers stain poorly for ATPase at alkaline pH values, whereas type II fibers stain intensely. Type II fibers can be further subdivided into subtypes A, B, and C by differential staining properties as the pH values are varied. Type I fibers are typically slow in contraction velocity and rely primarily on oxidative metabolism for energy. They are thus fatigue-resistant. Types IIA and IIB fibers are fast-twitch fibers that rely primarily on oxidative and glycolytic metabolic processes respectively. This classification system based on ATP staining correlates roughly with that of Peters, who grouped fibers as slow twitch (Type I), fast twitch oxidative-glycolytic (Type IIA), and fast twitch glycolytic

(Type IIB; Peter et al, 1972).

The development of a muscle fiber into its final subtype is influenced by many factors, including neurotropic hormones, sex hormones, thyroid hormones, and training (Kelly, 1983).

The mix of fiber types in a given muscle varies with the functional requirements (strength, velocity, and fatigue resistance) of that particular muscle. In lower animals, slowly contracting, postural muscles, for instance, are composed of a high percentage of type I (slow oxidative) fibers. By contrast, in man, most whole muscles are composed of a more homogeneous mixture of type I and type II fibers with specialized function of these muscles controlled by complex programming of the accompanying peripheral nerves (Gollnick et al, 1973). As will be discussed below, skeletal muscle fibers maintain a degree of plasticity. The fiber types, myosin composition, fatigue characteristics, and contractile properties may change in response to denervation, reinnervation, electrical stimulation and training (Barany and Close, 1971; Carraro et al, 1982; Costill, Fink, and Pollock, 1976; Gollnick et al, 1973; Sweeney et al, 1986).

The contractile properties of a muscle fiber are dependent, in large part, on the particular isoform of myosin composing the fiber. The subtypes of myosin can be separated biochemically or electrophoretically, allowing correlation of functional properties with the protein makeup of that fiber. The speed of contraction of a single muscle fiber, for instance, has been correlated directly with the concentration of the "fast" forms of heavy chain myosin present in that fiber (Reiser et al, 1985). Neural influence has been shown to be essential for expression of the slow forms of myosin light and heavy chains (Hoh and Yeoh, 1979).

Excitation-contraction coupling

In vivo, excitation and contraction of a muscle fiber begins with neural stimulation. at the neuromuscular junction, acetylcholine is released from the presynaptic nerve terminal and elicits depolarization of the motor end plate on the muscle fiber (Fig. 11-6). The action potential spreads quickly over the surface of the muscle fiber and ultimately results in contraction of that fiber. The action potential and subsequent contraction of all muscle fibers innervated by that particular nerve fiber are *all-or-nothing* events. Once the threshold for depolarization is reached in a muscle fiber, a maximal contraction or twitch occurs.

The depolarizing wave spreads over sarcolemma and enters the T-tubule system to quickly reach the myofibrils deep within the fiber. There are two components of the T-tubule system, the transverse and longitudinal portions. The transverse system is a series of invaginations of the sarcolemma, in contact with extracellular fluid, which runs perpendicular to the long axis of the muscle fibers and myofibrils. The longitudinal T-tubules, in contrast, are actually modifications of the intracellular sarcoplasmic reticulum and are oriented parallel to the myofibrils. The ends of the longitudinal T-tubules are vesicular and are termed *cisternae* or *terminal cisternae* (Eisenberg, 1983).

Depolarization of the cell membrane enters the transverse T-tubules and is propagated deep within the fiber. As depolarization approaches the longitudinal T-tubules, calcium is released from the cisternae and the actin-myosin cross-linking begins (Jobsis and O'Connor, 1966; Sandow, 1965). The first signs of mechanical activity usually follow within 1 to 2

milliseconds of the muscle action potential (Hodgkin and Horowitz, 1957).

An active calcium pump within the terminal cisternae returns the calcium to the longitudinal tubules rapidly following depolarization. The duration of an average calcium pulse is approximately 1/50 of a second (Guyton, 1979).

A defect in the control of the intracellular release and reabsorption of calcium during the excitation-contraction sequence is responsible for the catastrophic events of malignant hyperthermia. Dantrolene effectively inhibits calcium release from the cisternae, allowing cessation of the sustained muscle contraction.

Muscle contraction

The sequence of molecular events comprising contraction of the sarcomere is explained by the *sliding-filament theory* outlined in the laboratories of A. F. Huxley and H. E. Huxley in the 1950s (Hanson and Huxley, 1953; Huxley and Hanson, 1954; Huxley and Niedergerke, 1954; Squire, 1981). Observations of the sarcomere under light microscopy had previously revealed that the A-bands (the myosin-containing bands) of the sarcomere did not shorten during contraction. This led to the hypothesis that actin and myosin were actually separate filaments sliding past each other during sarcomere contraction. This hypothesis was later validated by electron microscopy, which demonstrated the crossheads of myosin molecules interdigitating with actin strands (Huxley, 1960).

To review the process, as calcium is released from the cisternae, it is bound transiently by troponin. Calcium effects a conformational change in the troponin-tropomyosin complex lying within the actin filament. As a result, the binding sites for the myosin cross bridges are exposed on the actin filament. Binding then occurs spontaneously as there is great natural affinity of the myosin heads for the actin-binding sites. Once binding has occurred, the myosin heads rock or flex to pull the actin molecule a short distance past the myosin molecule. This active ratchet mechanism of the myosin head occurs in the presence of ATP, which is hydrolyzed by an ATPase on the myosin head. The attachment, flexing, and reattachment of the myosin heads occurs repeatedly along the actin molecule, and results in the actin molecule sliding past the myosin molecule (Carlson and Wilkie, 1974). The speed of contraction and the concentration of ATPase differ with the different isoforms of myosin that are present in various skeletal muscle fiber types. A model summarizing the interaction of ATP hydrolysis with actin-myosin cross-bridge formation was introduced initially by Lymn and Taylor (1971).

Motor unit

Muscle fibers are grouped functionally into motor units (Buchthal and Schamlbruch, 1980). The fibers comprising a motor unit may be separated spatially within the whole muscle, but all muscle fibers in a motor unit are innervated by axons from a single nerve fiber. Generally, only one motor endplate and one axon innervate a skeletal muscle fiber. Exceptions are seen in the extraocular muscles and the intrinsic muscles of the larynx where multiple motor endplates and possibly polyneuronal innervation exist (Eisenberg, 1983; Guyton, 1979).

Activation of the neuron supplying a motor unit causes simultaneous contraction of all fibers within the motor unit. The response to activation is an all-or-nothing effort. The muscle fibers composing a motor unit share similar contractile properties and biochemical composition, that is, they are all of one fiber type. In fact, the phenotype of the fibers within each motor unit is determined largely by the motor neuron innervating those fibers (Kelly, 1983). Again, these generalizations may not be applicable to those rare muscles that demonstrate polyneural innervation.

Contractile properties

Contraction of skeletal muscle may be either *isotonic* or *isometric.* During isotonic contraction, the load or force applied to the muscle is constant and contraction produces muscle shortening and work (work = force x distance). Some of the earliest studies of contractile properties of whole skeletal muscles investigated the relation of velocity of contraction of a muscle to the load (force) applied to that muscle (Podolsky and Shoenberg, 1983). The force-velocity relationship resulting from these early studies is a well-known hyperbolic function describing the variation in contraction velocity with increasing load (Fig. 11-7). The relationship between force and velocity is described by the Hill equation (Hill, 1938).

During isometric contraction, the external length of the skeletal muscle fibers is held constant. Energy is expended developing tension within the fibers and heat is produced. There is no actual movement of a load and, technically, no work is done. On the molecular level, during isotonic contraction myosin cross bridges attach and reattach repeatedly to actin at the same binding site instead of sliding the actin filament past the myosin molecules (Huxley, 1974; Ruegg, 1981). Internal work is done in stretching the elastic component of the cross-bridge structure. The cyclic attachment and reattachment of myosin cross bridges continues as long as calcium and ATP are present.

A twitch is the response of skeletal muscle to a single suprathreshold stimulus. An isometric twitch is typically of shorter duration than an isotonic twitch because neither a change in shape of the fiber nor a change in inertia is required for the twitch. Under isometric conditions, if the frequency of successive twitches is increased so that the muscle does not completely relax between stimuli, *summation* of the tension is produced until a maximum, fused tension is reached. This state is termed *tetany* and the minimum frequency of stimulation required to produce tetany is called the *critical frequency* (Guyton, 1979). *Rigor* occurs when ATP stores are depleted and no energy is available to break actin-myosin cross links.

The length-tension relationship is illustrated in Fig. 11-8 and plots the maximum isometric tetanic force (tension) versus the length of the resting muscle fiber or fibers. As progressive passive lengthening of the muscle occurs, the actin and myosin filaments eventually overlap no longer and the contractile force produced approaches zero (Squire, 1981). The peak or maximum tetanic force is the maximum force a given fiber or muscle can produce, and occurs at that resting length (Lo) which allows optimum overlap and interaction of the actin and myosin filaments (Squire, 1981).

Experimentally, measurement of contractile properties such as maximum isometric

force, time to peak tension, relaxation time, power, and stiffness are valuable for the objective evaluation of skeletal muscle function. These properties may be compared following denervation, reinnervation, transplantation, training, and various forms of injury to quantify the effects of those interventions on the function of the muscle fibers. Isolation of single skeletal muscle fibers and chemical removal of the fiber membrane allows measurement of contractile properties on an isolated single muscle fiber (Julian, 1971). Subsequently, the protein composition of similar single fibers can be determined providing close correlation of muscle protein structure and function.

Plasticity of Skeletal Muscle

Denervation

As indicated, skeletal muscle fibers retain a certain plasticity and may be structurally and functionally altered in response to environmental changes. Denervation of skeletal muscle in the rat diaphragm, for instance, results in loss of the slow form of myosin light and heavy chains and a change in the phenotype of the myosin produced (Carraro et al, 1982). Reinnervation of fast muscle fibers with neurons originally innervating a slow muscle results in expression of the opposite slow myosin isoform in the reinnervated muscle (Barany and Close, 1971; Buller, Eccles, and Eccles, 1960). As discussed earlier, these variations in myosin structure ultimately produce changes in the function of the muscle fibers.

The changes in skeletal muscle contractile proteins that typically accompany denervation have been prevented experimentally in limb muscles by chronic electrical stimulation. Success in maintaining native myosin isoforms, however, depends on appropriate stimulation protocols, which vary for different fiber types (Nix and Dahm, 1987; Sweeney et al, 1986). Thus, to be optimally applied, native fiber types and contractile properties should be known. The potential for applying this technique clinically in denervated facial or laryngeal muscle underscores the need for more thorough understanding of normal and diseased skeletal muscle of the head and neck. Many unanaswered questions remain: When can a muscle no longer be reinnervated? Which donor nerves used in cross-reinnervation are an optimal match for the contractile properties of the recipient skeletal muscle? Which donor muscle optimally match the structural and functional characteristics of the native muscle when transplantation is an option? Can electrical stimulation of muscle enhance eventual reinnervation? Can electrical stimulation maintain a skeletal muscle on a long-term basis in the absence of neurotrophic factors?

Training

Athletic coaches have long recognized that muscle strength can be increased with high-resistance exercise. What is the mechanism of the strength gain? Does the strength increase occur because of changes in the contraction of the sarcomeres themselves, are new muscle fibers added, or are the types of fibers changed?

First, muscle power can increase early in strength training without measurable morphologic or biochemical changes in the muscle fibers. This occurs by a process of neural optimization in the programming of motor unit recruitment (Faulkner and White, 1990).

Physiologists have long recognized that force production is related to the cross-sectional area of the functional skeletal muscle. Data on single fibers and whole muscles indicate that there is probably no intrinsic difference in the maximum force produced by fast and slow muscle fibers; thus, an induced change in fiber types is not alone responsible for a gain in muscle power (Faulkner et al, 1980). In human whole muscle, the force produced is related much more closely to functional cross-sectional area than to age, sex, or fiber type (Saltin and Gollnick, 1983). With heavy resistance training, there is an increase in size of muscle fibers but probably no increase in the absolute number of muscle fibers present. Myofibrils are added during development to increase girth of muscle fibers and this same process occurs in response to high-resistance exercise (Goldspink, 1983). Thus, hypertrophy of muscle fibers, not hyperplasia, results in greater cross-sectional area and greater force. The capacity to develop tension per cross-sectional area has not been shown to increase during training, and it is hypothesized that the force produced by each myosin cross-bridging cycle is constant (Saltin and Gollnick, 1983).

However, the ratio of muscle fiber types composing a whole skeletal muscle may change to meet the demands of the activity. In the gastrocnemius muscles of trained sprinters and distance runners, for instance, there are a preponderance of fast (type II) fibers and slow (type I) fibers respectively. These proportions differ from the 50:50 mix of fast and slow fibers seen in nontrained control subjects (Costill, Fink, and Pollock, 1976). With cessation of the stimulus producing muscular adaptation, the changes reverse readily (Faulkner and White, 1990).

Clinical and Experimental Evaluation of Skeletal Muscle

A brief overview of the commonly used methods of evaluating muscle clinically and in the laboratory will be beneficial. This list and the accompanying descriptions are by no means exhaustive. To simplify, the techniques have been grouped according to those evaluating muscle structure and those evaluating muscle function.

Evaluation of skeletal muscle structure

Early investigators simply recorded muscle color and weight in response to experimental interventions. Red muscle was considered slow-twitch and white muscle was fast-twitch, generalizations that are now known not to be uniformly true. Documentation of changes in muscle mass in response to clinical and experimental stimuli continues to provide useful data.

Light-microscopic evaluation of skeletal muscle was introduced subsequently and can yield valuable information concerning fiber size, fiber atrophy, vascularity, and fibrosis. Augmentation of light microscopy wit special stains for neural elements detail the presence of nerve fibers and the morphologic degree of reinnervation. Electron microscopy (primarily transmission electron microscopy) played a central role in confirming the fine structure of the sarcomere (Huxley, 1960), and is currently used both clinically and experimentally in analyses of mitochondria and other subcellular organelles, myofibrilar structure, muscle spindles, and the neuromuscular junction in normal and diseased states.

Magnetic resonance imaging (MRI) of skeletal muscle allows measurement of muscle

size and flux of intracellular metabolites in vivo (Clark et al, 1988). A simple but important clinical application for MRI may be found in differentiating viable skeletal muscle from fibrotic, atrophic skeletal muscle in cases under consideration for reinnervation procedures. Intravital microscopy and radioactive microspheres are useful for evaluating the responses of intramuscular vasculature to various stimuli.

Histochemistry of skeletal muscle, introduced earlier in the section on fiber types, continues to be used for defining the various fiber types composing whole skeletal muscles. Histochemical stains have been developed for ATPase as well as for many of the other enzymes involved in oxidative metabolism (succinate dehydrogenase, lactate hydrogenase, (NAD-TR) and glycolytic metabolism (phosphopyrolase and alpha-glycerolphosphate dehydrogenase). However, the glycolytic enzyme markers are somewhat ambiguous and are usually excluded from fiber classification schemes (Gollnick and Hodgson, 1986). Histochemical stains are used to document metabolic changes in muscle fibers in response to experimental stimuli. They also provide a coarse correlation between metabolic make-up and measured contractile properties.

Biochemical assays of contractile proteins, noncontractile proteins, and oxidative enzymes have been used in evaluating muscle structure. Since the early 1970s gel electrophoresis has been used for separation of structural and contractile proteins and has, in large part, replaced the biochemical assays. Electrophoretic techniques have been refined extensively. Current techniques, for example, permit isolation and quantification of the light and heavy chain subunits composing the myosin molecule. Actin, troponin, and tropomyosin, can also be isolated electrophoretically. Important links between contractile protein composition and muscle fiber function have been documented by comparing the protein electrophoretic patterns with the measured contractile properties of identical skeletal muscle fibers.

Evaluation of muscle function

Direct observation continues to be a mainstay in the evaluation of skeletal muscle function. Facial motion may be videotaped and quantified, for example, providing an objective record of functional recovery over time (Burres and Fisch, 1986). Similarly, results of laryngeal reinnervation procedures have been documented by endoscopy, speech evaluation, and airflow assessment, all of which are essentially means of direct observation.

Experimentally, measurement of the contractile properties of skeletal muscle has been widely accepted as the most accurate indicator of muscle function. Force production, the velocity of muscle contraction, relaxation time, power, fatigue properties, and stiffness are among the variables that can be recorded reliably. Contractile properties can be measured in whole skeletal muscles, bundles of muscle fibers, and in single muscle fibers. Analyses have been performed in vivo and in vitro. Proper techniques and a clear understanding of when and where to apply them are essential to reliable and reproducible results (Brooks and Faulkner, 1988).

Electromyography and evoked electromyography have continued to be clinically important methods of skeletal muscle evaluation. Although direct measurement of contractile properties yields the most reliable quantitative data when obtainable, skeletal muscles cannot

be practically isolated in the clinical setting for measurement of contractile properties. Gaps in correlating electromyographic data with the morphology and contractile properties of skeletal muscle still exist, however. The role of electromyography may or may not increase as the morphologic and functional correlates of electromyographic patterns are more fully defined.