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A Review of the Technologies and Methodologies Used to Quantify Muscle-Tendon Structure and Function

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6.6 Summary

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6.1 Introduction

Muscle-tendon units are complex biological actuators able to generate considerable force to stabilize and/or move segments of the body and absorb energy imparted to the body. They are controlled through neural inputs and generate their forces by converting chemical energy into mechanical energy. Their mechanical behavior is directly linked to their macroscopic and microscopic structures and the properties of the specific proteins constituting these structures. Muscle-tendon units are highly adaptable, modifying their structure and protein forms in response to changes in environmental stimuli. Due to the integral role skeletal muscle plays in human function, an understanding of its behavior has been of interest for thousands of years. However, because of its complex organization of membranes, organelles, proteins,

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nerves, and vessels, and its versatility and adaptability, increases in our understanding of the detailed workings of skeletal muscle have often depended on the development of new technologies and methodologies. Much is still unknown about muscle-tendon structure and function and it is likely that further knowledge in this area will be achieved through technological innovations.

The purpose of this chapter is to provide detailed descriptions of muscle-tendon structure and function, and to summarize many of the technologies and methodologies employed over the years to unravel the intricate structures and functions of muscle-tendon units. While structure and function are directly related, for the sake of simplicity, they will be discussed separately. Muscle-tendon structure will be presented first, and a review of various approaches employed to study this structure will follow. Muscle-tendon function will be presented next, followed by a review of the approaches employed to study function.

6.2 Muscle-Tendon Structure

In this section, a detailed description of the structural organization of a muscle-tendon unit is presented. The description of the structural organization of muscle begins at the level of the whole muscle and proceeds to the smaller subunits, concluding with the proteins constituting the myofilaments. Membrane systems, neural, vascular, and connective tissue networks are described. The variability in muscle fiber structures and how this variability has led to various fiber-type naming schemes will then be discussed.

Skeletal muscle exists in a variety of shapes and sizes. It is composed of many subunits arranged in an organized, but complex manner (see Fig. 6.1). Additionally, muscles connect in series to tendons, are innervated by nerves, and supplied with vascular networks. A whole muscle is surrounded by a strong sheath called the epimysium, and divided into a variable number of subunits called fasciculi. Each fasciculus is surrounded by a connective tissue sheath called the perimysium. Fascicles may be further divided into bundles of fibers (or muscle cells) surrounded by a connective tissue sheath called the endomysium.[8,26,51,54,88,91,108,109,110] Beneath the endomysium are two additional membranes, the basal lamina and the plasmalemma.[26,88,96] The orientation of fibers relative to the line of action of the muscle-tendon complex is referred to as the pinnation angle. In humans, the pinnation angle ranges from 0 to 25°.[88,121] Muscle may be classified as fusiform (or spindle), penniform, bipenniform, triangular, rectangular (or strap), and rhomboidal. Fibers attach at both ends to tendon or other connective tissue. Muscle fibers contain mitochondria, multiple nuclei, ribosomes, soluble proteins, lipids, glycogen, and satellite cells. Fibers are cylindrical, with their diameter ranging from 10 micrometers (μm) to 100 μm (smaller than the size of a human hair).[88] They may be a few millimeters (mm) or many centimeters (cm) in length. Fibers are subdivided radially into myofibrils having diameters of approximately 1 μm. Myofibrils are divided longitudinally into sarcomeres and radially into myofilaments. A saromere is defined as the region between Z-lines (defined below). Sarcomeres have a rest length of about 2.0 to 3.0 μm. Myofilaments are often classified as either thick or thin filaments.

Thick filaments are composed primarily of myosin molecules. Myosin accounts for approximately 55% of the myofibril volume. It is composed of two heavy chains and four light chains. Two light chains are associated with each heavy chain. The two heavy chains are identical, whereas the light chains vary within different fiber types. Each myosin molecule is rod shaped with two adjacent globular heads at one end. The myosin molecule structure has been defined in terms of two general regions: the light meromyosin (LMM), and the heavy meromyosin (HMM). The LMM represents part of the tail. The HMM contains the two heads, and the remaining part of the tail not considered part of the LMM. HMM is further divided into subfragment 1 (S1) and subfragment 2 (S2) (see Fig. 6.1). Myosin molecules are about 160 nanometers (nm) long (myosin rod is 140 nm and head is 15 nm) and 2 nm in diameter.[8,26,108,110] Myosin molecules are arranged to give a total thick filament length of 1.55 μm and 12 to 15 nm diameter.[80] There are approximately 100 axial locations along the thick filament, separated by 14.3 nm where myosin heads exist. The number of myosin molecules terminating at each axial repeat location is still controversial. Most of the evidence has been interpreted as suggesting three myosin ends per axial repeat distance. Each

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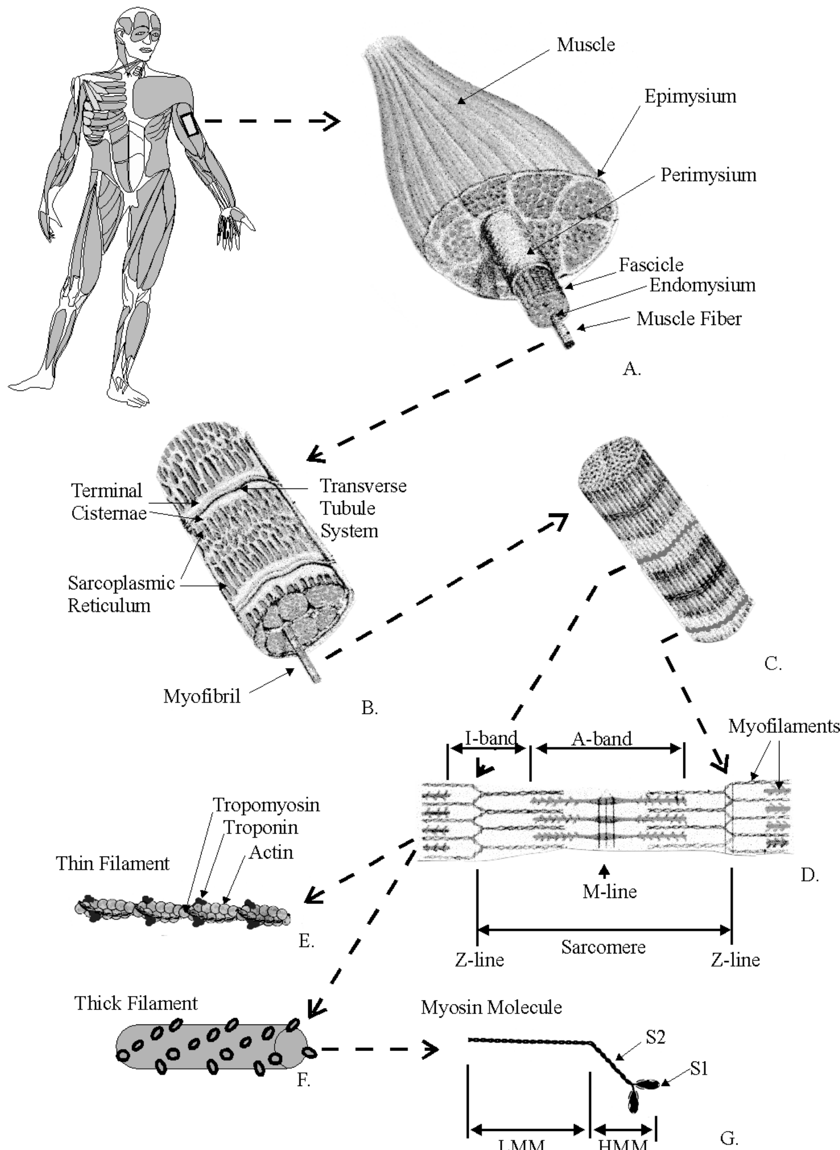

FIGURE 6.1 Illustration of the strucutral organization of muscle. A whole muscle is shown in A, a muscle fiber in B, a myofibril in C, a sarcomere in D, a thin filament in E, a thick filament in F, and a myosin molecule in G.

thick filament contains approximately 300 myosin molecules (assuming three myosin ends per axial repeat location).[26] At least 8 proteins in addition to myosin are affiliated with the thick filament: C-protein, H-protein, M-protein, myomesin, M-creatine kinase, adenosine monophosphate (AMP) deaminase, skelemin, and titin.[8,26,88,110]

Thin filaments are composed primarily of actin, tropomyosin, and troponin. Thin filaments are approximately 1 μm long and 8 nm in diameter. Each thin filament contains about 360 actin monomers. Each actin monomer consists of a single polypeptide chain.[8] Actin monomers polymerize to form a double helix pattern with a repeat spacing of 5.5 nm.[8,88] Because of symmetry and the spherical shape of the actin monomers, there exists a groove on either side of the helix chain. Each groove is filled by a series of tropomyosin-troponin complexes, each spanning a length of seven actin monomers (41 nm in length). There is one troponin molecule, approximately 26 nm long, for each tropomyosin molecule.

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The tropomyosin molecule forms an α-helical coiled coil structure. The troponin molecule can be further divided into troponins C, I, and T.[88,108]

Thick and thin filaments are oriented parallel to one another within a sarcomere and typically have a zone of overlap (see Fig. 6.1). The region containing the thick filaments is referred to as the anisotropic or A-band, approximately 1.55 μm in length. The region containing the thin filaments with no overlap with the thick filaments is termed the isotropic or I-band. The 0.16 μm region in the center of the A-band that has no thin filament overlap is called the Helle* or H-zone. In the middle of the A-band is a region called the middle or M-line. The M-line is composed of a connective tissue network binding the thick filaments. At the end of each sarcomere is a dense protein zone called the Z-line** (also referred to as the Z-disk or Z-band).[42,91] The Z-disk is composed of a connective tissue network binding the thin filaments. It contains the proteins α-actinin, desmin, filamin, and zeugmatin.[26] Thin filaments are attached at the Z-disk but are free to interdigitate with the thick filaments at their other ends. When viewed in cross section through the zone of overlap between thin and thick filaments, a hexagonal lattice appears with one thick filament surrounded by six thin filaments. The spacing between thick filaments is 40 to 50 nm.[80] The spacing between thick and thin filaments is 20 to 30 nm.[8]

The muscle fiber contains two distinct membranous systems: the transverse tubular system (T-system or T-Tubule system) and the sarcoplasmic reticulum (SR) (see Fig. 6.1).[8,26.80,88] The T-system is part of the plasmalemma and makes a network of invaginations into the cell near the Z-line in amphibian muscle and near the junction of the A- and I-bands in mammalian muscle.[26] No part of the contractile machinery is further than 1.5 μm from a T-tubule.[72] Two terminal cisternae (part of the SR) run parallel to the T-system to form a triad.[96] The T-system is separated from the terminal cisternae by a distance of about 16 nm but connects to the terminal cisternae via numerous feet.[72] The SR traverses longitudinally from the terminal cisternae.

In addition to the structures mentioned above, vascular, neural, and connective tissues play important roles in muscle function. Muscles have a rich supply of blood vessels that supplies the oxygen needed for oxidative metabolism. Capillary networks are arranged around each fiber with the capillary densities varying around different fiber types.[80]

The basic neuromuscular element is called the motor unit. It consists of a single alpha motoneuron and all the muscle fibers it innervates. The number of fibers per motor unit is variable, ranging from just a few in ocular muscles requiring fine control, to thousands in large limb muscles.[23,80] Fibers from a given motor unit tend to be dispersed throughout the muscle cross section rather than clumped together in one region. Oxidative fibers tend to occur in greater percentages deeper in the muscle compared to glycolytic fibers which have higher percentages in the perphery.[89] The structure of the neuromuscular junction can vary significantly between different species, between different fiber types of the same species, and during the course of development. In general, the nerve terminal ending on a muscle fiber contains vesicles 50 to 60 nm in diameter. These vesicles contain acetylcholine (Ach), adenosine triphosphate (ATP), a vesicle-specific proteoglycan, and a membrane phosphoprotein, synapsin. Approximately 15% of the nerve terminal volume is taken up by mitochondria. The nerve and muscle membranes are not in direct contact. The synaptic space is approximately 50 to 70 nm wide and contains acetylcholinesterase (AchE). The muscle membrane contains nicotinic Ach receptors.[26] The muscle membrane has several folds in the regions of the nerve endings to increase the transmitter reception area eightfold to tenfold.

Muscles have extensive connective tissue networks located both in parallel and in series with the fibers. Myofibrils appear to be attached transversely at periodic adhesion sites. The protein titin spans the distance between Z-lines and the middles of the thick filaments.[8] Muscle fibers are connected in series with tendons. The primary structural unit of tendon is the collagen molecule. Type I collagen consists of three polypeptide chains coiled together in a right-handed triple helix held together by hydrogen and covalent bonds.[43,120] Collagen molecules are organized into long, cross-striated fibrils that are arranged into bundles to form fibers. Fibers are further grouped into bundles called fascicles, which group together

*German for "light."

**From Zwischen-Scheibe, meaning "interimdisk."

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to form the gross tendon. Elastic and reticular fibers are also found in tendon along with ground substance (a composition of glycosaminoglycans and tissue fluid). In an unstressed state, collagen fibers take on a sinusoidal appearance, referred to as a crimp pattern.

Although the general structures (i.e., actin and myosin filament lengths and their lattice arrangement) are similar among vertebrate muscle fibers, there are differences in the regulatory proteins of the myosin and troponin, the extensiveness of membrane networks, and the number of mitochondria and other organelles. These variations have functional consequences that led to the development of a variety of naming schemes to identify fibers with specific structural and functional properties (e.g., red/white, fast/slow, oxidative/glycolytic, types I/IIa,b,c, and SO/FOG/FG).[19,20,23-25,29,94,107] The myosin molecule appears in various isoforms.[56,79,105] These isoforms exhibit different amino acid sequences, ATPase activity, and affinity for calcium.[99] The troponin C protein may vary in its sensitivity to calcium. There are differences in the membrane networks. The T-system may be twice as extensive in one fiber compared to another. Mitochondrial density also varies among fibers.[26]

6.3 Approaches Used to Study Muscle-Tendon Structure

Our understanding of the complex structural organization of muscle-tendon units described above has come from keen observations and the development of a variety of technical tools and novel methodologies. The first recorded scientific medical studies were undertaken by the Greeks around the 6th century B.C.[9] However, most of the studies conducted prior to the 17th century, which contributed to our understanding of muscle structure, were based on gross dissections and involved identifying muscles, tendons, nerves, and the vascular network. Since then, advances in mathematics, chemistry, physics, and genetics have played a major role in identifying and characterizing muscle-tendon structure.

Microscopy has been used extensively to study muscle. Lenses were first used to magnify objects around 1600 A.D.[104] Microscopes, in which various arrangements of flat, concave, and convex lenses are used to magnify images, were introduced around the beginning of the 17th century. Microscopy has developed into a highly technical field utilizing a variety of illuminating approaches.

Light microscopy was the first technique employed to study muscles and other biological tissues. Leeuwenhoek (1632–1723) was one of the first great biological microscopists. He manufactured hundreds of microscopes which he used to observe many biological tissues. Unfortunately, much of his expertise in tissue preparation and illumination was lost throughout the 18th and 19th centuries. Much of the work in light microscopy conducted then centered around correcting for artifacts and aberrations through matching glass, refractive media, and improving lens manufacturing.[104] Muscle appears transparent when viewed using normal light microscopy, and therefore it is often stained prior to viewing. A variety of stains have been used to provide the contrast necessary to identify different organelles and gross structures.[104] In addition, the light used to illuminate the specimen has been manipulated in various ways to cause refraction and interference patterns that allow different structures within muscle to be visible.

Dark-ground, phase contrast, interference, and polarization microscopy identify regions of different refractive indices, but they accomplish this based on fundamentally different approaches. While most living, non-stained biological tissue is transparent when investigated with normal light microscopy, different regions of a cell have different refractive indices. In dark-ground microscopy, light is passed through the specimen at rather oblique angles so that the direct light beam passes to the side of the objective.[104,114] The only light entering the objective comes from refracted light. Regions of high refractive index appear bright against a black background as they reflect the light to the eyepiece or viewing port. Phase contrast microscopy makes use of the relative phase differences in light passing through different regions of the tissue having different refractive indices. These phase differences are converted to changes in light intensity in the image plane.[114] Interference microscopy splits the illuminating beam into two beams. One beam passes through the specimen and the other beam passes around it.[8] The two beams are recombined before the objective. Light passing through high refractive index tissue is slowed down, phase shifted, relative to light passing around the tissue. The interference pattern that results indicates different protein-dense zones. If the proteins within a region which give rise to its refraction index are

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not homogeneously distributed, then the refractive index will depend on the plane of polarization of light. A polarization microscope takes advantage of this property. Basically, a polarizer located at the condenser causes a single plane of light to illuminate the specimen. An analyzer located after the specimen allows a single plane of light to pass to the objective. The alignment of polarizer and analyzer is variable, but they are usually set at right angles.[104,114] The object stage can rotate relative to the plane of polarization. The terminology commonly used to describe sarcomere anatomy is largely the result of muscle observations made under polarization microscopes. When viewed with a polarization microscope, specific zones of a muscle fiber appear darker than other zones. The dark zones have dense protein bands causing the plane of polarization of light to be strongly rotated. These zones have been labeled anisotropic or A-bands. Other zones are less protein dense and rotate the plane of polarization of light weakly. These zones have been labeled isotropic or I-bands.[8,51] The Z-band is also observed to be anisotropic while the H-zone in the middle of the A-band appears relatively isotropic.

The use of light as an illuminating medium has inherent resolution limitations. Basically, the best resolving power of a microscope is equal to about 0.6 times the wavelength of the electromagnetic radiation used to illuminate the specimen. The use of short wavelengths provides better resolution (e.g., 475 nm wavelength blue light provides better resolution than 700 nm wavelength red light, and X-rays with wavelengths of about 0.1 nm are better than visible light). The attainable resolving power of light microscopy is about 200 nm and that of electron microscopy is about 0.1 nm.[104] Based on the various structural dimensions presented previously, it is evident that light microscopy could be used to distinguish Z-lines with 2 to 3 μm separation distances, but could not be used to distinguish between myofilaments having spacings of 20 to 50 nm.

Due to resolution limitations inherent in using light, further resolution of muscle structure using microscopy depended on the development of electron microscopy (EM). The theoretical concept of an electron microscope was proposed in the 1920s.[104] The concept was formulated from the ideas that particles have wave properties and a magnet can be used to focus a beam of electrons similar to the way a lens focuses light. By the 1940s many countries were making transmission electron microscopes. Following the development of transmission electron microscopy (TEM), scanning electron microscopy (SEM) was developed. SEM utilizes the reflected electrons to make an image of the object in contrast to recording the transmitted electrons in TEM. It has the advantage of providing greater topographical information about the specimen than TEM. However, SEM provides a very low contrast signal, and its utility has relied on the development of computer algorithms for amplifying, averaging, and processing the signals in other ways.

Conventional preparation of a specimen for EM involves fixation by cross-linking agents, dehydration, embedding in resin, sectioning, and staining with electron-dense heavy metals. One obvious drawback to this technique is that the tissue is dead and harshly handled prior to viewing. Nonetheless, electron microscopy has revealed much about muscle and tendon structure. It revealed that the banding pattern in skeletal muscle arises from interdigitation of sets of filaments. Thin filaments were observed to connect to the Z-line and make up the I-band. Thick filaments were observed to compose the A-band with thick and thin filaments having a region of overlap. High magnification electron micrographs showed connections between thick and thin filaments in the overlap zone. These connections were referred to as cross-bridges. EM, in combination with techniques such as freeze-fracture and protein purification, has provided much of what we know about the structure of contractile proteins, the membrane networks, and the neural innervation zones.[8,26,108]

In addition to microscopy, muscle has been examined using diffraction techniques. A diffraction pattern arises whenever a beam of electromagnetic radiation passes through a narrow slit or a small hole. The hole or slit causes the beam to spread and acquire regions of destructive interference such that a banding pattern or a series of concentric rings results. When monochromatic light is used to illuminate muscle, the striation pattern within muscle gives rise to an optical diffraction pattern. The distance between fringes can be used to calculate sarcomere length.[8] X-rays having wavelengths of about 0.1 nm can be used to illuminate muscle and create a diffraction pattern that can be used to calculate the spacing between filaments, the spacing between cross-bridges, and even the spacing between actin monomers

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(5.5 nm).[8,88,110] This technique in conjunction with EM has been used extensively to reveal much of what we know about the molecular structure of muscle. A major advantage of diffraction studies is that they can be applied to thin sections of living tissues.

A variety of other techniques have been used to identify the molecular structure of muscle. Thick and thin filament composition were determined through extraction/aggregation studies. Selective extraction of A- and I-bands with salt solutions revealed that thick filaments are composed mainly of myosin and thin filaments are composed mainly of actin. Evidence indicating that the cross-bridges represent the HMM end of myosin came from aggregation studies.[109] When LMM aggregated it gave a smooth structure. When intact myosin molecules aggregated they formed a large number of projections. Different myofibrillar isoforms have been identified using peptide finger printing, monoclonal antibodies, and the application of recombinant DNA procedures.[26] Fluorescence techniques are now used to study protein distribution within a cell.[68]

Like muscle, tendon structure has been determined using a variety of techniques. Chemical techniques have been used to determine its protein and molecular components. Light microscopy and tissue staining techniques have revealed the vascular, neural, and fiber structures within tendon as well as the locations of fibroblast cells. Polarization microscopy in combination with special stains has been used to isolate the fibrous elements of collagen, elastin, and reticulin. Electron microscopy has been used to determine the organization of collagen molecules.[43,120] A summary of some of the approaches used to study muscle-tendon structures is given in Fig. 6.2.

Summary of Approaches Used to Determine Muscle-Tendon Structures

Approach Employed	Examples of Structures Identified
I. Gross Dissection	I. Muscle-tendon attachments and gross, architecture, blood vessels, nerves
II. Microscopy	II. Cell structures
A. Light	A. Microscopic cell structures
1. Normal with stains	1. Muscle cell organelles, membranes
2. Dark-ground	2. Regions of different refractive index
3. Phase-contrast	3. Regions of different refractive index
4. Interference	4. Regions of different refractive index
5. Polarization	5. A- and I-bands, Z-lines
B. Electron	B. Molecular structures
1. TEM	1. Actin and myosin, cross-bridges
2. SEM	2. 3 dimensional images of membrane vesicles and contractile proteins
III. Diffraction	III. Spacing between structures
A. Monochromatic Light	A. Sarcomere lengths
B. x-ray	B. Axial repeat spacing of myosin heads, myofilament spacing
IV. Chemical	IV. Chemical composition
A. Extraction combined with electron microscopy	A. Contractile proteins and sub-fragments
B. Antibody labeling combined with electron microscopy	B. Contractile proteins and sub-fragments
C. Electrophoresis	C. Molecular weight of proteins

FIGURE 6.2 A summary of various approaches that have been used to study muscle-tendon structure.

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6.4 Muscle-Tendon Function

This section provides descriptions of the functions performed by the individual structures identified in the previous section, the processes involved in energy supply, the processes involved in converting chemical energy into mechanical force, and the factors that affect muscle-tendon performance.

Functions of Specific Structures

Nuclei dictate cell material and distribution. Like cell managers, they keep structures organized. Nuclei communicate with other nuclei within a cell to maintain some consistency of regulation.[88] They also exhibit local regulatory control, especially at locations near the sites of neural innervation. The amount and type of protein to be produced are defined by a nucleus and carried out by the ribosomes in response to mRNA. Ribosomes are granules of ribonucleoprotein. Protein synthesis can be up- or down-regulated fairly quickly, providing muscle the ability to adapt. The speed, strength, and endurance properties of the cell are dictated by the proteins comprising the cell.

Mitochondria located in the cytoplasm produce ATP through oxidative metabolism. ATP is the energy source used for all cell functions (e.g., protein synthesis, ion transport, repair, and force production). Mitochondrial density depends on function. It may be as high as 20% by volume for highly oxidative fibers.[41,42]

Other important substances contained in the cytoplasm are glycogen, lipids, and enzymes. Glycogen and lipids are sources of ATP. Glycogen is a polymer of linked glucose which can be used as an immediate source of ATP through anaerobic glycolysis performed by soluble enzymes. Lipids serve as a second energy source, but require oxygen for their metabolism. Thus, they are most prevalent in cells with high mitochondrial density.[88]

The extensive membrane network of muscle cells performs several functions. The endomysium provides structural support for the muscle fiber and the neural and vascular tissues interacting with it. The basal lamina appears to play a role in injury repair. Complete repair can occur rapidly if the basal lamina is intact to provide a scaffold for regeneration.[26,54,88] The basal lamina also communicates with the nerve to signal it where to innervate the muscle fiber if denervation has occurred. The plasmalemma, T-system, and SR function as semi-permeable barriers, conduits for electrical signal propagation, filters, and calcium storage centers. The plasmalemma acts as a filter by requiring a certain number of receptors on its surface to be stimulated before changing its membrane permeability and conducting the electrical signal of the nerve into the cell. The T-system provides the conduit for rapid transmission of electrical activity to the inner regions of the cell. The SR stores and releases calcium ions which are essential for force production and relaxation.

Sarcomeres are the basic units of shortening and force generation and thus have numerous structures of functional importance. The Z-line is a highly organized structure that interconnects the thin filaments in a very precise array. The M-line is presumed to be responsible for binding the thick filaments and maintaining them in a hexagonal pattern when viewed in a transverse plane. The thick filaments contain myosin molecules which perform several tasks. The HMM portion of myosin is often referred to as the cross-bridge because it is the structure that reaches out and binds to actin during contraction. The HMM-LMM interface is flexible, allowing the S1 portion of HMM to project out about 55 nm[8] to reach a thin filament. S1 contains binding sites for two light chains: ATP and actin. Thin filaments play an equally important role in force production. Actin monomers have binding sites compatible with regions of the S1 portion of myosin. These binding sites are normally covered by tropomyosin during rest conditions. However, in the presence of calcium, troponin C, which is sensitive to calcium ion binding, causes troponin I to produce a conformational change in tropomyosin which then exposes the myosin binding sites. Troponin T functions to regulate troponin-tropomyosin binding. Two final structures that may have functional importance are nebulin and titin. Nebulin runs parallel to the actin filaments and may function in length determination during assembly. Titin is a relatively large elastic filament that stretches from M-line to Z-line. It provides passive elasticity and helps to keep the A-band centralized.[8]

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Processes Involved in Energy Supply

All the processes involved in cell maintenance and force production rely on the availability of ATP and thus a discussion of the processes involved in ATP synthesis and supply is relevant. ATP is the universal energy source for all cells. Energy comes from splitting ATP into adenosine diphosphate (ADP) and inorganic phosphate (Pi). ATP is normally bound to Mg in skeletal muscle, but myosin can hydrolyze ATP and release its energy. This reaction is very slow in isolation, about 0.01 ATP/sec, but in the presence of actin this rate increases to 4.5 ATP/s and in actual skeletal muscle this process proceeds at a rate of about 6.3 ATP/myosin head/sec.

The body provides several means of supplying ATP to muscle.[73,74] The amount of ATP present in living muscle can provide enough energy for only about eight muscle twitches.[91] Obviously the body provides some means of quickly replenishing ATP. The pathway most commonly used during the onset of physical activity combines ADP with phosphocreatine (PCr) to produce ATP and creatine (Cr). This reaction is often referred to as the Lohmann reaction and can take place in either direction. However, the equilibrium constant for the reaction favors the production of ATP by a factor of about 20. PCr must be present in the muscle for the Lohmann reaction to proceed toward ATP production. Muscle maintains a small reserve of PCr, but not enough to supply the amount of ATP needed for sustained activities. In fact, the amount of PCr stored in muscle tissue can provide enough ATP to sustain several hundred twitches.[8] This is much greater than what the stores of ATP can supply, but still not sufficient to supply the energy demands placed on the body during daily activities.

Aerobic phosphorylation and anaerobic glycolysis provide additional pathways for ATP production. Anaerobic glycolysis can be considered a process in itself or a precursor to oxidative phosphorylation. Whether or not oxidative phosphorylation occurs depends on oxygen availability to the muscle cell and the content of cytochromes and myoglobin present within the cell. During anaerobic glycolysis, which takes place in the cytoplasm, a series of reactions break down glucose to form two pyruvic acid, two hydrogen, and four ATP molecules. Anaerobic glycolysis utilizes two ATP molecules to breakdown glucose, hence the net yield is two ATP molecules. The pyruvic acid and hydrogen molecules generated from anaerobic glycolysis enter the mitochondria where the Kreb's cycle (also referred to as the tricarboxylic acid or TCA cycle) takes place. For each pyruvic acid molecule entering the Kreb's cycle, three CO_2 molecules, five hydrogen molecules, and one ATP molecule are formed. The hydrogen atoms released from both the Kreb's cycle and anaerobic glycolysis enter an electron transport system (ETS) by combining with nicotinamide-adenine dinucleotide (NAD). Aerobic oxidative phosphorylation will occur at this stage if sufficient oxygen is available to meet the supply of hydrogen transported to the mitochondria via NAD. If the oxygen supply is not sufficient, then NADH reacts with the pyruvic acid to form lactic acid. Lactic acid can accumulate in the muscle and cause fatigue. At some point, usually during a recovery period, the lactic acid is cleared from the muscle and carried to the liver where it is synthesized into glucose. Provided oxygen is available, a total of 32 ATP molecules along with CO_2 and water are produced from the NADH. Energy is needed to transport the two hydrogen molecules generated during anaerobic glycolysis from the cytoplasm into the mitochondria. This process utilizes one ATP molecule per hydrogen molecule transferred. Thus the net yield of ATP per glucose molecule from aerobic metabolism is 34. The aerobic processes are much more efficient than anaerobic glycolysis acting alone, which yields only two ATP molecules per glucose molecule. Also no lactic acid is formed; only CO_2 and H_2O are produced.

Processes Involved in Force Development and Transmission

Muscles generate force by converting chemical energy into mechanical force in response to electrical signals received from a motoneuron. The basic functions of force development and shortening are initiated through the processes of excitation-contraction coupling. These processes are initiated when a peripheral nerve action potential arrives at a muscle fiber's synaptic cleft (or motor end plate). This action potential may result from signals sent from the brain or through reflex pathways (discussed more in the section titled "Effects of an Integrated Multiple Muscle System"). Signals are passed from nerve to muscle by chemical transmitters. When an electrical signal arrives at a motor end plate, the membrane allows

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calcium to flow into the cell.[27] The increased intracellular calcium ion concentration causes vesicles located on the membrane to release acetylcholinesterase (Ach) which diffuses across the synaptic cleft and binds to specific receptors on the muscle membrane. If sufficient binding takes place, then the permeability of the muscle membrane changes (reaches threshold).[54]

The number of receptors that must be stimulated to cause these changes varies for different fiber types. Permeability changes cause sodium ions to enter the cell and potassium ions to leave the cell. The membrane depolarizes, becoming less negative inside the cell. The signal, or action potential, is propagated in both directions along the length of the muscle fiber. An action potential is always the same for a given cell. The cell depolarizes in an all-or-none response once a sufficient stimulus is achieved. After the action potential, there is a refractory period in which the cell cannot be activated again. The refractory period is necessary to prevent back flow of impulses.

Excitation of the muscle membrane spreads inward through the T-system which communicates this excitation to the SR. The SR then releases calcium ions along the length of the fiber. The calcium binds with troponin C which causes troponin I to create a conformational change in tropomysin which exposes an actin binding site for myosin.[80,96] Two calcium receptors must be stimulated in slow oxidative fibers to remove the inhibitory effect of Troponin I, while only one is required in fast glycolytic fibers. The S1 portion of a neighboring myosin molecule binds with the actin and develops force. If the force developed by all bound myosin heads is greater than the external force applied to the muscle or muscle-tendon unit, then the muscle will shorten. The muscle will lengthen or remain at a constant length if the force is less than the external force, or equal to the external force, respectively. Force will continue as long as there are bound myosin heads. However, in the presence of ATP, the myosin adenosine triphosphatase (ATPase) will hydrolyze the ATP and the acto-myosin bond will be broken. Myosin ATPase activity is approximately three times faster in fast-glycolytic fibers than it is in slow oxidative fibers.[59,86] Myosin will continue to form new bonds with actin as long as there is sufficient calcium to bind with troponin C. Once the action potential stops the Ca^{+2} is pumped back into the SR. The rates of myosin ATPase activity and membrane system release and uptake of Ca^{+2} regulate the rate of force development and relaxation.

Factors Affecting Muscle-Tendon Performance

The force developed by the muscle and actually transmitted to the bones via its associated tendons depends on the neural input, the muscle-tendon architecture, the muscle kinematics, the muscle composition of different fibers, the contraction history, and the feedback from various proprioceptors.

Effects of Neural Input

The level of force generated by voluntary contraction of skeletal muscle is controlled by at least two neural mechanisms, motor unit recruitment and modulation of the firing rate of active motor units (rate coding). It is generally accepted that motor units are recruited in an orderly manner consistent with the size principle of Henneman et al.[64,65] According to Henneman, the excitability or threshold level at which a motor unit is recruited is inversely related to the diameter of the motoneuron. Thus the participation of a motor unit in graded motor activity is dictated by the size of its neuron. It appears that slow fibers are innervated by small, low threshold, slow conducting motor nerves. Fast fibers are innervated by larger, higher threshold, faster conducting motor nerves. Thus, slow fibers are recruited first, followed by fast fibers. Studies conducted by other researchers have supported this finding.[3,18,30,49,50,61] Rate coding allows force regulation through summation of the force developed by single twitches. There is a frequency of stimulation above which twitch responses become fused and fibers generated their maximal force. Below the fusion frequency, fibers generate submaximal forces which vary relative to the stimulation frequency.[18,67]

Effects of Muscle-Tendon Architecture

At the level of the gross muscle, the physiological cross-sectional area (PCSA) is most commonly used to indicate a muscle's strength, fiber length, orientation, and type to indicate its maximum velocity of

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shortening.[95,117] PCSA is calculated by taking the product of muscle mass and the cosine of the pinnation angle, and dividing by the product of fiber length and muscle density. It is important to note that mass alone does not dictate strength, but rather mass and fiber length do so. A muscle with short fibers oriented at some angle relative to the axis of the muscle-tendon complex will generate greater maximum force than a muscle of similar mass that has longer and fewer fibers. Because muscle fibers are composed of serial arrangements of sarcomeres, fiber length affects shortening velocity. Longer fibers have faster shortening velocities, provided the fiber types are similar.

Tendon length and compliance affect muscle-tendon performance.[1,44,45,101,122] A long compliant tendon protects a muscle from injury during sudden imposed stretches. It also transmits muscle force slowly. Short, rigid tendons transmit force rapidly, but provide little protection to the muscle and little potential for storage of elastic strain energy.

Effects of Muscle-Tendon Kinematics

Considerable evidence has been compiled over the years indicating that the amount of force that a muscle can produce depends on its length.[10,21,22,29,52,57,102] Specifically, the force is proportional to the overlap of thick and thin filaments. The fiber length determines the amount of thick and thin filament overlap which determines the number of cross-bridges capable of attaching and developing force. There is an optimal range of muscle fiber length over which the fiber can produce its greatest force. This range occurs at fiber lengths causing the thick and thin filaments to overlap such that all cross-bridges may be active, without overlap of actin filaments from adjacent sarcomeres. At longer fiber lengths not all cross-bridges may contribute to force generation and the force declines. At shorter lengths actin filaments from adjacent sarcomeres begin to interfere with each other and the force also declines. Muscle can also generate passive force. In general, passive force increases gradually from 100 to 130% of rest length and stiffens with increased length. At rest length up to 150%, the deformation is reversible, after which it becomes plastic. The passive properties of muscle may be due to the large molecule titin and membrane structures.

Muscle velocity also affects the force developed. It has been shown that as muscle force increases, the rate of muscle shortening decreases in a hyperbolic fashion.[69,71,82] If muscle is stretched it generates a force greater than its isometric force. Unlike the force-length relationship, the force-velocity relationship has not yet been explained on a precise anatomical basis.

Effects of Muscle Composition

The type of muscle fiber comprising a gross muscle affects the muscle's performance. As discussed previously, myosin molecules in fast and slow twitch skeletal fibers have different ATPase activities.[59,99,103,105] These differences have been correlated with the different shortening velocities that exist between these fiber types.[11,59,103] There are also differences in the troponin C protein in fast and slow twitch fibers. Only one Ca^{+2} site has to be filled to trigger contraction in slow fibers compared to multiple sites in fast fibers.[99] The extent of the T-system varies among different types of muscle fibers. In mammalian muscles, fast twitch fibers have T-systems that are about twice as extensive as those of slow twitch fibers.[80] This property gives rise to faster relaxation rates in fast twitch fibers. Mitochondrial density varies. Fibers relying on oxidative metabolism have greater numbers of mitochondria compared to fibers relying on anaerobic metabolism. These fiber types have the potential to develop force for greater duration compared to glycolytic fibers.

Effects of Contraction History

The contraction history of a muscle-tendon complex can act to reduce or enhance performance relative to how the complex would perform during a standard isometric or concentric action. Fatigue acts to reduce the force that the entire muscle can generate.[6,15,40,55,60,115] However, the mechanisms of fatigue may vary. Basically, anything that inhibits the normal processes of excitation-contraction and coupling described above may cause fatigue. Some of the possible sites where fatigue may be initiated include the central nervous system, the motor end plates, the cytoplasm if pH changes occur, the membranes, and the contractile proteins.

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The term *enhancement* has been used in the literature to describe two different effects: (1) elastic energy storage, and (2) force potentiation, an increased force above that of a similar contraction initiated from rest.[4,5,84,113] The first of these effects is related to muscle-tendon elastic properties. The second effect is less understood. However, for both forms of enhancement, the magnitude of the effect depends on several factors. First, for any enhancement to occur a stretch/shortening cycle (eccentric contraction followed by a concentric contraction) must take place. Other factors of relevance are the time delay between the two contraction modes (referred to as coupling time), stretch velocity, initial muscle length prior to stretch, and the amplitude of stretch.[7,16,17,38,39,58,116] The exact mechanisms responsible for enhancement have not been isolated. Storage of elastic strain energy in the tendon and series elastic components of muscle have been suggested as possible sources of the improved mechanical efficiencies reported during certain activities.[2,4,5,28,35,46,113]

Like elastic strain energy, force potentiation is a complex issue. Force potentiation created by a stretch/shortening cycle may be due in part to greater force developed by each cross-bridge attached. There appears to be an optimal eccentric force or amplitude of stretch, below which the magnitude of the force potentiation increases with increased stretch amplitude, and above which it begins to decrease.[4,5] If cross-bridges are stretched too far, then they break and the increased force is lost.

Effects of an Integrated Multiple Muscle System

Under normal conditions muscle-tendon units do not act in isolation. Muscles are influenced by their own actions, which generate specific feedback signals and the signals generated by other muscles and tissues. A motoneuron pool originates in the anterior horn of the spinal cord. Input to a motoneuron pool comes from afferent impulses sent from peripheral receptors, the Renshaw system, and from higher brain centers. These signals may be transmitted along alpha, gamma, or beta neurons.

Feedback to a muscle comes primarily from muscle spindles, and Golgi tendon organs. A muscle spindle is a fusiform capsule attached at both ends to the muscle fibers and arranged in parallel to the fibers. Inside this capsule 2 to 25 are intrafusal fibers. These fibers can contract like extrafusal fibers, but are distinguished because they have centrally located nuclei. At the end of each fiber bundle are two groups of afferent nerves, Ia and II (Ia nerves are larger). Ia afferent nerves connect directly to the motoneuron pool of the muscle and provide excitatory signal. They also connect disynaptically to antagonist muscles to provide inhibitory signals. Group II afferent nerves connect disynaptically to the original muscle only and provide excitatory signals. Ia and II afferent nerves modify their discharge rates when their endings are elongated either by stretching of the muscle or shortening of spindle fibers. Ia afferent nerves are sensitive to length and rate changes, whereas II afferent nerves are primarily sensitive to small length changes.[14,36]

The Golgi organ is located in the aponeurosis and extends from a tendon into the muscle. It has nerve endings sensitive to force. The Golgi organ has a fusiform shape. It is about 650 microns long and 50 microns in diameter. It is innervated by Ib afferent nerves which can generate an inhibitory effect on muscle and a facilitating effect on antagonist muscles, both through disynaptic connections. Renshaw cells, which reside completely in the anterior horn of the spinal cord, are collateral cells that generate negative feedback to nearby neurons. Their role in motor control is not really known.[14]

Muscle-tendon units within the body attach to bones and generate forces to produce joint torques and movement. Muscle-tendon attachment locations directly affect a muscle's potential for moving a limb and generating torque. A muscle-tendon unit with an attachment site relatively far from the joint center will have a mechanical advantage (or expressed more appropriately, less of a mechanical disadvantage since muscle-tendon units usually have severe mechanical disadvantages relative to the external loads they must oppose) compared to a muscle-tendon unit attaching closer to the joint center. However, the latter muscle will have an advantage over the first muscle in producing joint velocity. Thus, relative to performance, joint strength and speed of movement are dictated by the properties of all muscle-tendon units crossing the joint and the locations of their skeletal attachment sites. The musculoskeletal system has considerable redundancy and numerous muscles can create torques about a given joint. These muscles are activated to produce a given torque based on some control scheme that is not understood and likely

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varies among people and complexities of tasks. Further, there appear to be differences among people in their abilities to realize the full force generating potentials of their muscles and to coordinate the activation of multiple muscles. These differences translate into differences in gross movement performance. A summary of the functions of various muscle-tendon structures is given in Fig. 6.3.

Summary of the Functions of Various Muscle-Tendon Structures

Structure	Function
I. Whole Muscle-Tendon Unit	I. Generate force to stabilize and/or move limb segments. Absorb energy from external sources to reduce loads to other tissues. Store elastic energy for potential reutilization.
II. Fibers	II. Normal cell functions
A. Nuclei	A. Specify DNA sequence for cell proteins
B. Mitochondria	B. Supply ATP through oxidative phosphorylation
C. Ribosomes	C. Produce cell proteins
D. Motor end plate	D. Nerve-muscle fiber interface, filter inputs
E. Membrane Systems	E. Ion barrier, electrical signal conductor
F. Satellite Cells	F. Generate new fibers after injury
G. Sarcomere	G. Basic contractile element
1. Thick Filament	1. Stationary filament
a. Myosin	a. Force development
1) HMM	1) The cross-bridge
a) S1	a) Binding site for actin, site of ATP hydrolysis
b) S2	b) Support for S1
2) LMM	2) Backbone of myosin
2. Thin Filament	2. Translate along thick filament to allow muscle length change.
a. Actin	a. Contains binding sites for myosin
b. Tropomyosin	b. Controls exposure of myosin-sensitive binding sites on actin.
c. Troponin	c. Controls tropomyosin configuration
1) - I	1) Inhibit actin-myosin binding
2) - C	2) Calcium sensitive receptor, controls Troponin-C action.
3) - T	3) Regulate Troponin-Tropomyosin binding
3. M-line	3. Maintain thick filaments in register
4. Z-line	4. Maintain thin filaments in register
5. Titin	5. Provide series elasticity, possibly regulate length assembly
III. Motor Unit	III. Basic neuromuscular element
IV. Tendon	IV. Transmit muscle force, store elastic energy

FIGURE 6.3 A summary of the functions of various muscle-tendon structures.

6.5 Approaches Used to Study Muscle-Tendon Function

The approaches used to study muscle-tendon function are numerous. The review in this section is not intended to be inclusive, but rather to provide a general overview of the wide variety of techniques that have been employed to study those factors affecting muscle-tendon performance described in the previous section. Specifically, studies of the interaction between muscle mechanics and energy utilization, force and neural input, force and length, force and velocity, general performance and architecture, general performance and muscle composition, general performance and contraction history, and general

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Muscle-Tendon Function	Approach Used to Study Function
Muscle mechanics and energy utilization	- isolated muscle preps, muscle stimulation, ergometers, and calorimeters - isolated muscle preps, muscle stimulation, gas analyzers, conversion from oxygen consumption to chemical energy utilization - same approach as above but applied to intact muscle - isolated muscle preps, ergometer, muscle stimulation, quick freeze techniques and chemical analysis - intact muscle, force or pressure transducer, NMR
Force and ... Rate coding	- electrical simulation of varying frequencies, force transducer - voluntary contractions, force transducer, electrodes for recording frequency of muscle activation
Recruitment	- indwelling electrodes to record single motor unit activity, force transducer, gradual increase in voluntary contraction effort - voluntary effort of varying intensity, muscle biopsies to determine motor units depleted of glycogen
Length	-isolated muscle preps, light microscopy, force transducer - intact muscle, extensometer, goniometer or videography, force transducer or dynamometer
Velocity	- isolated muscle preps, lever systems with adjustable loads or electromagnetic ergometers, optical displacement transducers, stimulators - intact muscle, dynamometers

Summary of Approaches Used to Study Muscle-Tendon Function

FIGURE 6.4 A summary of various approaches used to study muscle-tendon function.

performance and multiple muscle interactions are discussed. A summary of the approaches used to study muscle tendon function is given in Fig. 6.4.

Muscle Mechanics and Energy Utilization

A variety of methods have been used to determine the energy utilized by a muscle to generate force under various conditions. One approach used for isolated muscle preparations involves placing the muscle in a calorimeter, attaching one end of the muscle to a force transducer or ergometer, activating the muscle, and recording the chemical energy used by the muscle, the work performed, and the heat liberated.[19,48,69] This is the most precise and accurate method, but it is not very applicable to studying muscle *in vivo*. An alternative approach is an indirect method in which the oxygen consumed by the muscle is recorded. The chemical energy used by the muscle is estimated based on the relationship between ATP synthesis and oxygen utilization. This method has been used to study both isolated muscle preparations and muscles acting *in vivo*.[12,13,32,87,90,111]

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Summary of Approaches Used to Study Muscle-Tendon Function (Continued)	
Muscle-Tendon Function	**Approach Used to Study Function**
General Performance and ... Muscle Architecture	- dissection, imaging techniques, force transducers, dynamometers
Tendon Architecture	- mechanical testing systems, extensometers, optical tracking devices
Muscle Composition	- same tests as force-length and force-velocity, combined with tests to identify fiber types
Contraction History — Fatigue	- electrical stimulation to differentiate central versus peripheral mechanisms - fura-2 and fluorescence microscopy to determine if stimulus is reaching inner cell - pH probes - caffeine administration to determine if cross-bridge is fatigue site - stiffness measurements to determine if force loss is due to reduction in force/cross-bridge or number of cross-bridges
Enhancement	- same as force-velocity, but comparing results from muscle or muscle groups contracting with and without a stretch-shortening cycle - same as mechanics and energetics, but comparing results from muscle or muscle groups contracting with and without a stretch-shortening cycle
Multiple Muscle System	- buckle force transducer to measure force directly - predict force based on model and inputs from EMG, goniometers or videography - estimate force using an inverse dynamics analysis and input from force plates and videography

FIGURE 6.4 (Continued)

Other approaches have quantified the amount of ATP, inorganic phosphate (Pi), and phosphorylcreatine (PCr) before and after muscle activation. These measurements can be used to determine the chemical energy utilized. In one such approach, an isolated muscle is attached to an ergometer and caused to contract. After the contraction the muscle is immediately frozen and the above quantities measured using chemical techniques.[35,118] In a second approach, nuclear magnetic resonance imaging is used to quantify the concentrations of free ATP, PCr, and Pi.[8,118] This method may be used to study muscle *in vivo*, but the signal intensity is very low and multiple trials and signal averaging techniques are required.

Force and Neural Input

Rate coding and recruitment are neural activation characteristics that can regulate muscle force production. Force transducers, neural stimulators, and recording electrodes are the common instruments used to investigate these neural factors although some chemical techniques have also been employed.[3,37,56,64,66,81,92,100] The effect of rate coding has been investigated by stimulating a muscle at different frequencies via its nerve and recording the force developed. Voluntary contractions have also

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been performed with recording electrodes used to monitor the stimulation frequency over time. The effects of recruitment and the order of motor unit recruitment have been investigated by placing small electrodes within a muscle and recording the electrical activities of single motor units as a person voluntarily contracts the muscle and generates increasingly greater force. Motor units are activated and deactivated in a specific order.[100] The idea of a rank order of recruitment has been supported in several other studies.[18,49,50,61]

Glycogen depletion studies have also been performed to identify which fiber types are involved in different intensities of muscle activation. In these studies, a person utilizes a muscle to produce a given level of force. A muscle biopsy is taken and those fibers depleted of glycogen are identified and classified. In general, oxidative fibers are recruited first, followed by the glycolytic fibers.

Force and Length

The sliding filament theory of muscle length change was developed from results of phase-contrast and interference microscopy[75,76,78] while the mechanisms responsible for the parabolic force-length relationship were demonstrated using X-ray diffraction and electron microscopy.[77] Results from phase-contrast and interference microscopy indicated that the A-band of a muscle fiber does not change length during muscle length change whereas the I-band does. This led to the proposal that filaments slid past one another during muscle length changes. Electron microscopy later identified the individual filaments and the cross-bridges connecting them. Electron microscopy also revealed that cross-bridges could only move about 100 to 140 Å while the length changes observed in the fiber were on the order of 30% of the original length.

This led to the proposal that cross-bridge cycling must occur and that the cross-bridges act as individual force generators. Support for this idea came with the recording of both force and length changes. It was shown that the greatest force occurred when there was optimal overlap of thick and thin filaments, and that the active force decreased in a linear fashion as the length was increased until the thick and thin filaments no longer overlapped, at which time the active force was zero.

Studies of the force-length behaviors of intact muscles have also been performed. These studies rely on force transducers or dynamometers to quantify muscle force or joint torque. Muscle length changes are recorded using video analysis techniques, extensometers, and/or limb displacement measurements combined with musculoskeletal models.

Force and Velocity

The force-velocity relationship of muscle has been derived based on numerous studies of both isolated and intact muscles.[70,71,82,83,106,112] Isolated muscles were stimulated and allowed to shorten while opposed by different load magnitudes. The resistive loads were created with weights and lever systems or electromagnetic devices. The results demonstrate the hyperbolic decrement in velocity for increased load. The experiments conducted on intact muscle involved joint dynamometers which can control either the joint torque or joint angular velocity. The results from intact muscle do not always match those of isolated muscle, but the general trend of decreased velocity for increased force or torque does apply.[112]

General Performance and Muscle-Tendon Architecture

The architectural arrangement of muscle fibers within a muscle affects the amount of force exerted along the axis of the muscle, and the range of muscle lengths over which the muscle can generate force.[23,52,117] Our understanding of the effects of muscle architecture on muscle performance has come from comparative studies of the force-length and force-velocity profiles of muscles that have different architectures. Muscle models have also been used to investigate architectural effects.[52,53,95,98,122]

Tendon structural properties are generally characterized using a mechanical testing system to stretch the tendon while the force and deformation are recorded.[119] These data have been used to determine the tendon's compliance and energy storing capacity.[1,43,44,101]

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General Performance and Muscle Composition

The relative compositions of fiber types comprising a muscle affect the muscle's maximum shortening velocity, rate of force development, relaxation rate, fatigue resistance, rate of energy utilization, and power output.[47] Studies illustrating this fact have involved both isolated muscles and intact muscles.[24,31,85,86,111,112] Isolated muscle studies were done by attaching a homogeneous muscle or muscle fiber to an ergometer and recording the force time profile following stimulation. Following the mechanical testing, the muscle was examined via one of the techniques discussed previously to classify the fiber type.[20,25] Different fibers were shown to have different rates of force development and relaxation, different maximum shortening velocities, and different fatigue resistance properties.

Studies of intact human muscles have relied on muscle biopsies to quantify the relative percentage of each fiber type within a muscle combined with joint testing to quantify the torque and power produced by that muscle, and the muscle's fatigue resistance. Testing is usually performed using a single joint and a joint dynamometer or a specific movement such as cycling.[31,56,112] Differences in the rates of energy utilization have also been demonstrated among fiber types.[85,86,118] The techniques used for this determination are the same as those presented in the section on "Muscle Mechanics and Energy Utilization."

General Performance and Contraction History

The techniques used to isolate the mechanisms responsible for muscle fatigue include electrical stimulation, mechanical stiffness measures, and a variety of chemical methods. If a decrement in force results from some mechanisms outside the muscle, then electrical stimulation can be used to elicit a greater force output. For example, if force output during a maximum isometric contraction declines but can be returned to the initial value through external stimulation to the muscle, then the site of fatigue occurred outside the muscle. The site of fatigue within a muscle is difficult to isolate and probably varies depending on the contractile conditions. Fibers have been injected with fura-2 which binds with calcium and can be tracked using digital imaging fluorescence microscopy. This technique has been used to determine whether the excitation signal is carried into the center of the cell and pH probes have been used to determine whether cellular pH changes occur to cause fatigue.

Caffeine has been used to determine whether fatigue is due to insufficient activation of the contractile proteins. Caffeine has the effects of increasing the release of calcium from the SR, reducing the uptake of calcium by the SR, and increasing the troponin C sensitivity to calcium. Thus, if upon administration of caffeine the force increases, then the site of fatigue does not reside in the contractile proteins. Muscle stiffness measurements have been performed in an attempt to determine whether force decrements are due to a decrease in the number of cross-bridges actually generating force or the actual force per cross-bridge. In practice, combinations of these various techniques are used to isolate the site of muscle fatigue.

Force enhancement has been studied in both isolated and intact muscles.[7,16,17,28,38,39,46,84,113] The instruments employed in both cases are similar to those already discussed. Isolated muscle studies involve neural stimulation and muscle force measurements via use of a force transducer or ergometer. Intact muscle studies involve either isolated joint testing with a dynamometer or the determination of gross movement efficiencies by quantifying oxygen consumption and the mechanical work done using force plates and/or some form of motion analysis system. The degree of muscle force enhancement is determined by comparing muscle force or efficiency between muscle actions with and without a stretching-shortening cycle.

General Performance and Multiple Muscle Systems

Historically, three basic approaches have been utilized to predict muscle force *in vivo*. The first approach is direct and relies on some device such as a buckle force transducer to directly monitor the force developed by the muscle. This approach has been used in animal models and to a very limited extent in humans. The second approach is indirect and relies on measurements of specific muscle parameters (e.g., activation levels, kinematics, and architecture) and a suitable mathematical muscle model to compute the forces in

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individual muscles.[63] The third approach is also indirect, and involves first solving the inverse dynamics problem to determine intersegmental loads (i.e., forces and moments), then utilizing a musculoskeletal model which predicts the behavior of individual muscles when certain criteria like objectives and cost parameters are specified.[33,34,63,97,122]

The instrumentation utilized to obtain the data needed for these approaches includes force plates, electromyography, accelerometers, buckle force transducers, goniometers, and dynamometers. Unfortunately, all of these approaches have limitations and the results obtained are far from consistent for even the most basic human movements. Clearly, our modeling approaches are crude and likely neglect many factors that are critical to the behaviors of muscle-tendon units *in vivo*.

6.6 Summary

In summary, muscle-tendon units involve complex arrangements and interactions of a variety of macroscopic and microscopic structures. A number of techniques have been utilized to identify these structures. Many of these techniques have inherent limitations which necessitate the use of multiple techniques to confirm structural identification. Thus, our understanding of muscle-tendon structure comes from cross-checking the results of many different types of experiments. The contractile characteristics of a whole muscle depend on both gross muscle architecture and the properties of the fibers comprising the muscle. All vertebrate skeletal muscle fibers are similar in their structural arrangement of actin and myosin, but have variations in their membrane structures, density of their mitochondria, specific protein isoforms, and possibly myofibril packing density. These differences, at the molecular level, cause differences in fiber contractile characteristics (i.e., fiber force, maximum shortening velocity, and resistance to fatigue).

At the level of the whole muscle, differences exist among muscles in their arrangements of fibers and percentages of each fiber type. Variations in fiber properties and gross muscle structure mean that different muscles have different contractile characteristics and functions. Our understanding of muscle-tendon function, like muscle-tendon structure, has developed from the findings obtained from use of a variety of technological and methodological approaches. These findings are not always consistent and thus multiple approaches are often required to adequately test various theories of muscle-tendon function.

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