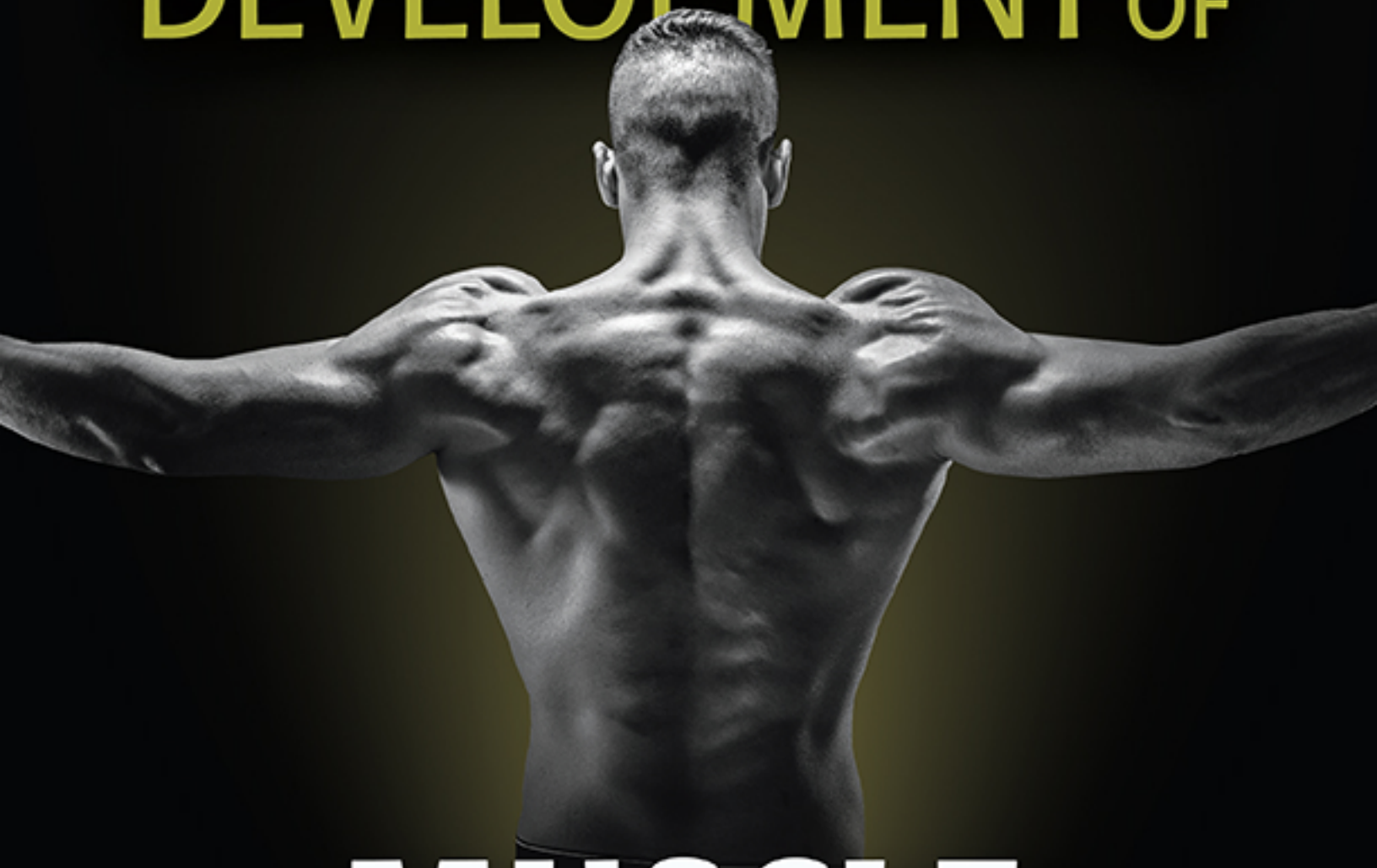
SECOND EDITION

SCIENCE AND DEVELOPMENT OF



MUSCLE HYPERTROPHY

Brad Schoenfeld

Science AND Development OF Muscle Hypertrophy

Second Edition

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To my father, may he rest in peace, for instilling the scientific method in me for as long as I can remember. You pushed me to learn, to pursue higher education, and to become a scholar. Wish you were around to see the fruits of your efforts. This is for you; I know it would have made you proud.

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PREFACE

This book truly has been a labor of love.

I had envisioned writing an evidence-based text on muscle hypertrophy since my days as a graduate student in exercise science. At the time there were a plethora of consumer-oriented books describing programs for building muscle. However, they all relied largely on anecdote to make recommendations; none extensively delved into the actual science of the topic. A more scientific approach was clearly needed for the masses. In 2016, my vision became reality with publication of the first edition of *Science and Development of Muscle Hypertrophy*.

Much has transpired since the release of the book's first edition. For one, research on muscle hypertrophy has skyrocketed. Thousands of new studies have been published, helping to further our understanding as to what makes muscle grow and how to best go about optimizing muscle development. Moreover, feedback and the perspective of time have allowed me to see ways in which the original text could be improved and expanded. Ultimately, I determined that a revision of the original text was warranted.

I am thrilled to present the second edition of *Science and Development of Muscle Hypertrophy*. The text has been completely updated, with inclusion of more than 30% new content. In addition to containing extensive discussion of new research findings and their practical implications to muscle building, I have added two new chapters of importance: one that delves into the methods employed to measure muscle growth and another that evaluates various advanced training practices commonly employed to enhance hypertrophy. Further, 10 new sidebars highlight specific topics of interest to gaining lean mass.

A few words of note about the book in general: While the writing is geared toward master's level students in exercise-related disciplines, the majority of the text should be accessible to anyone with a fundamental understanding of the principles of exercise science. The first two chapters are the most scientifically technical, and will require some background in exercise physiology and biomechanics to fully appreciate the complexities and challenges faced when attempting to draw inferences as to the underlying mechanisms of what drives hypertrophic adaptations. However, even if you do not possess a strong scientific background, much information can be gleaned from at least reading through these chapters to familiarize yourself with basic concepts and terminology.

Despite its scientific basis, the overall focus of the book is on the applied aspects of muscle development. As such, each chapter contains "key points" that summarize take-home messages and their practical applications. There also is an entire chapter (chapter 8) devoted to synthesizing the literature in an evidence-based fashion to create customized hypertrophy-oriented programs.

In sum, I hope you agree this is the most complete resource on the market for bridging the gap between science and practice to optimize muscle development.

Knowledge is power; learn and thrive.

ACKNOWLEDGMENTS

First and foremost, to Roger Earle, for envisioning this project and providing all the necessary resources to ensure its quality. I am thankful for your trust in me writing the book and for your continual guidance throughout the publication process. Without your efforts, this book would not have come to fruition. I am eternally grateful.

To Shawn Donnelly, for effectively and efficiently managing the development of this project so that everything ran smoothly. Your efforts were greatly appreciated.

To Grant Tinsley, Mike Israetel, Cody Haun, Henning Wackerhage, James Krieger, Adam Sharples, Alan Aragon, Bret Contreras, Mike Roberts, and Andrew Vigotsky, for providing input on the book. Your insights helped to improve its breadth and ensure its accuracy.

Finally, to my past and present students, who perpetually inspire me to learn and grow and to be the best I can be in my field. Your personal development and success are what drive me to keep doing what I am doing and are part of what make my life so fulfilling.

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Hypertrophy-Related Responses and Adaptations to Exercise Stress

chapter 1

To comprehend the many factors related to maximizing skeletal muscle hypertrophy, it is essential to have a foundational knowledge of how the body reacts and adapts to exercise stress. This chapter reviews the structure and function of the neuromuscular system and the responses and adaptations of the neuromuscular, endocrine, paracrine, and autocrine systems. Although these systems are discussed separately, they are integrally connected; their interactions ultimately mediate lean tissue growth.

Neuromuscular System

A detailed discussion of the complexities of muscle hypertrophy requires a fundamental understanding of the neuromuscular system—in particular, the interaction between nerves and muscles that produces force to carry out human movement. Although a thorough exploration of the topic is beyond the scope of this book, this section provides a general overview of concepts that are referenced in later chapters. Those interested in delving further into the subject are advised to seek out one of the many textbooks specific to exercise physiology.

Structure and Function

From a functional standpoint, individual skeletal muscles are generally considered single entities. However, the structure of muscle is highly complex. Muscle is surrounded by layers of connective tissue. The outer layer covering the entire muscle is called the *epimysium*; within the whole muscle are small bundles of fibers called *fasciculi* that are encased in the *perimysium*; and within the fasciculus are individual muscle cells (i.e., fibers) covered by sheaths of *endomysium*. The number of fibers ranges from several hundred in the small muscles of the eardrum to over a million in large muscles such as the gastrocnemius. In contrast to other cell types, skeletal muscle is *multinucleated* (i.e., contains many nuclei), which allows it to produce proteins so that it can grow larger when necessary. Individual muscle fibers can span lengths of up to approximately 600 millimeters (23 inches) and their volumes can exceed those of typical mononucleated cells by more than 100,000-fold (202).

Skeletal muscle appears striped, or *striated*, when viewed under an electron microscope. The striated appearance is due to the stacking of sarcomeres, which are the basic functional units of myofibrils. Each muscle fiber contains hundreds to thousands of *myofibrils*, which are composed of many *sarcomeres* joined end to end. Myofibrils contain two primary protein filaments that are responsible for muscle contraction: *actin* (a thin filament) and *myosin* (a thick filament), which comprise approximately 50% of the protein content of a muscle cell (53). Each myosin filament is surrounded by six actin filaments, and three myosin filaments surround each actin filament, thereby maximizing their ability to interact. Additional proteins, including titin, nebulin, and myotilin, are present in muscle to maintain the structural integrity of the sarcomere or aid in regulating muscular contractions, or both. Figure 1.1 shows the sequential macro- and microstructures of muscle tissue.

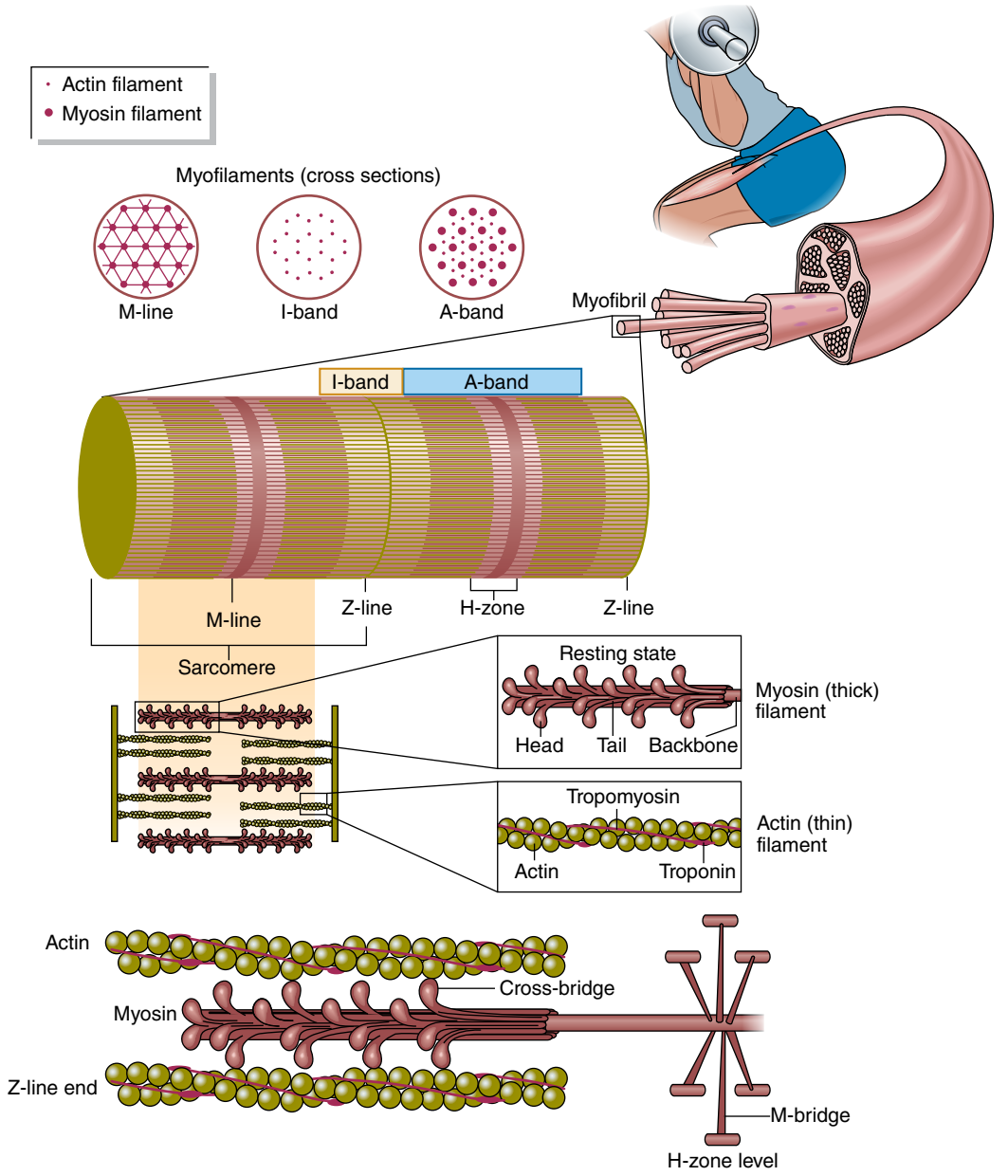

FIGURE 1.1 Sequential macro- and microstructures of muscle.

Motor Unit

Muscles are innervated by the nervous system. Individual nerve cells associated with muscular actions are called *motor neurons*. Motor neurons consist of three regions: a cell body, an axon, and dendrites. When a decision is made to carry out a movement, the axon conducts nerve impulses away from the cell body to the muscle fibers, ultimately leading to muscular contraction. Collectively, a single motor neuron and all the fibers it innervates is called a *motor unit* (figure 1.2). When a motor unit is innervated, all of its fibers contract; this is known as the all-or-none principle.

Sliding Filament Theory

It is generally accepted that movement takes place according to the *sliding filament theory* proposed by Huxley in the early 1950s (97). When a need to exert force arises, an action potential

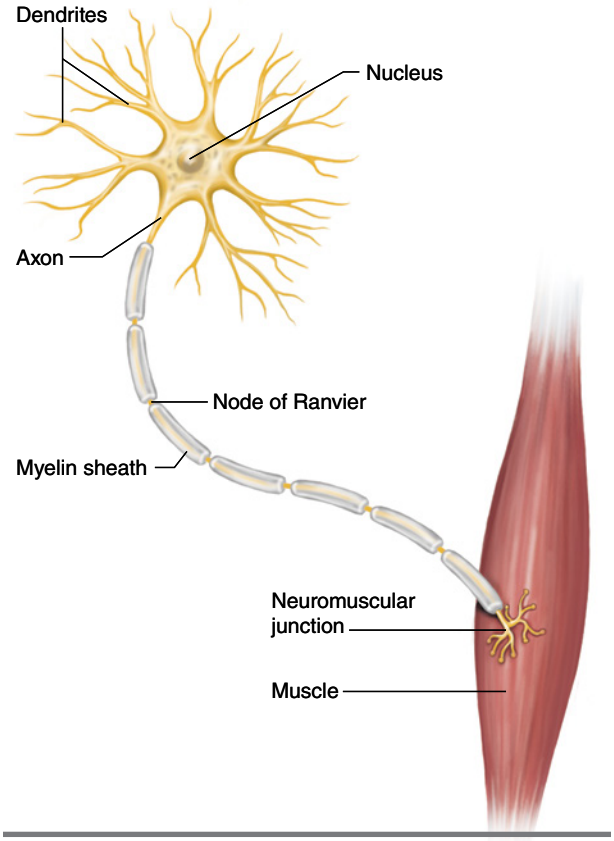

FIGURE 1.2 A motor unit.

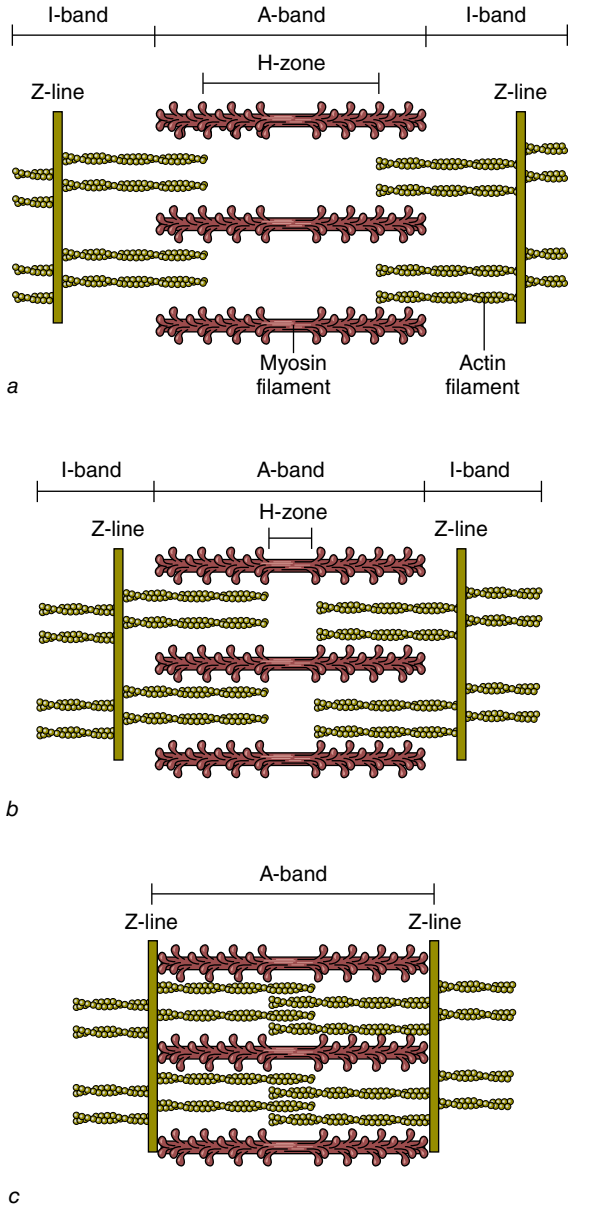

FIGURE 1.3 Contraction of a myofibril. *(a)* In stretched muscle, the I-bands and H-zone are elongated, and there is low force potential as a result of reduced crossbridge–actin alignment. *(b)* When muscle contracts (here, partially), the I-bands and H-zone are shortened. Force potential is high because of optimal crossbridge–actin alignment. *(c)* With contracted muscle, force potential is low because the overlap of actin reduces the potential for crossbridge–actin alignment.

travels down the nerve axon to the neuromuscular junction, where the neurotransmitter acetylcholine is released across the synaptic cleft and ultimately binds to the muscle fiber's plasmalemma. This depolarizes the muscle cell, causing calcium to be released from the sarcoplasmic reticulum. Calcium binds to troponin, which in turn moves tropomyosin from actin binding sites so they are exposed to myosin. Assuming sufficient ATP to drive muscular contraction, the globular myosin heads bind to exposed actin sites, pull the thin filament inward, release, and then reattach at a site farther along the actin filament to begin a new cycle. The continuous pulling and releasing between actin and myosin is known as crossbridge cycling, and the repeated power strokes ultimately cause the sarcomere to shorten (figure 1.3).

Fiber Types

Muscle fibers are broadly categorized into two primary fiber types: *Type I* and *Type II*. Type I fibers, often referred to as slow-twitch fibers, are fatigue resistant and thus well suited for activities requiring local muscular endurance. However, peak tension takes time—approximately 110 ms—to achieve in these fibers, thereby limiting their ability to produce maximal force.

Type II fibers, also known as fast-twitch fibers, serve as a counterpart to Type I fibers. They can reach peak tension in less than half the time—just 50 ms—thereby making them better suited for strength- or power-related endeavors. However, they fatigue quickly and thus have limited capacity to carry out activities requiring high levels of muscular endurance. The greater myoglobin and capillary content in slow-twitch fibers contributes to their higher oxidative capacity compared to fast-twitch fibers. Table 1.1 summarizes the characteristics of the primary muscle fiber types.

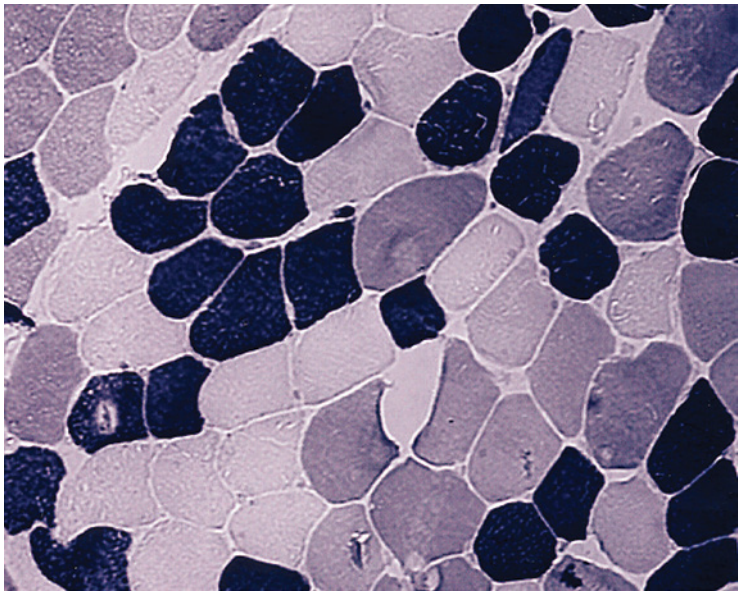

FIGURE 1.4 A photomicrograph showing Type I (black), Type IIa (white), and Type IIx (gray) muscle fibers.

Reprinted by permission from W.L. Kenney, J.H. Wilmore, and D.L. Costill, *Physiology of Sport and Exercise,* 5th ed. (Champaign, IL: Human Kinetics, 2012), 37.

Muscle fiber types are further distinguished according to the predominantly expressed isoform of myosin heavy chain; they are referred to as Type I, Type IIa, and Type IIx (236). Several other similar forms (commonly called *isoforms*) have been identified, including Ic, IIc, IIac, and IIax (figure 1.4). From a practical standpoint, the c isoform typically comprises less than 5% of human muscle and thus has minimal impact on total cross-sectional area.

On average, human muscle contains approximately equal amounts of Type I and Type II fibers. However, a large interindividual variability exists with respect to fiber type percentage. The quadriceps of elite sprinters have been shown to have a predominance of Type II fibers, whereas quadriceps of elite aerobic endurance athletes are primarily composed of Type I fibers.

TABLE 1.1 Characteristics of Muscle Fiber Types

	Type I	Type IIa	Type IIx
Size of motor neuron	Small	Medium	Large
Contraction time	Slow	Moderately fast	Fast
Force production	Low	Moderate	High
Resistance to fatigue	High	Moderate	Low
Mitochondrial density	High	Moderate	Low
Oxidative capacity	High	High	Low
Glycolytic capacity	Low	High	High
Capillary density	High	Moderate	Low
Myoglobin content	High	Moderate	Low
Glycogen stores	Low	High	High
Triglyceride stores	High	Moderate	Low

That said, a wide variability in these percentages exists even at the top levels of sport. World champion hurdler Colin Jackson was determined to have a fast-twitch fiber population of 71% in the vastus lateralis, with an extremely high abundance (24%) of the pure Type IIx isoform (230); in comparison, research shows elite Danish sprinters possess 57% fast-twitch fibers in the vastus lateralis, with just approximately 11% of the Type IIx variety (14). Moreover, certain muscles are predisposed to higher percentages of a given fiber type. For example, the endurance-oriented soleus contains an average of more than 80% Type I fibers; the more strength-oriented triceps brachii contains approximately 60% Type II fibers (50).

Many experts claim that all Type II fibers are inherently larger than Type I fibers. However, there is evidence that women often display a larger cross-sectional area of Type I fibers than of Type IIa fibers (236). Research does indicate that the oxidative properties of a fiber, rather than fiber type, influence muscle size. Specifically, the cross-sectional area of glycolytic Type IIx fibers is significantly greater than that of the more oxidative Type I and Type IIa fibers. It has been speculated that the smaller size of high-oxidative myofibers is an evolutionary design constraint based on the premise that muscle tissue has a limited capacity to hypertrophy and increase oxidative capacity at the same time (236). This is consistent with the hypothesis that competition exists between the turnover rates of structural (myofibrillar) proteins and those involved in metabolism (i.e., mitochondrial proteins), which is seemingly mediated by interactions between signaling pathways involved in either the synthesis or degradation of the respective muscle proteins (236).

Another often-proposed assumption is that Type II fibers are primarily responsible for exercise-induced increases in muscle size. This is principally based on studies showing that Type II fibers experience superior growth compared to Type I fibers after regimented resistance training (1, 40, 43, 111, 201, 217). When considered as a whole, the literature indicates that the growth capacity of Type II fibers is approximately 50% greater than that of Type I fibers (6), although substantial interindividual variability is seen in the extent of fiber type–specific hypertrophic adaptation (111). There also is evidence that the rate of muscle protein synthesis is elevated to a greater extent in the primarily fast-twitch human vastus lateralis muscle (approximately 50% to 60% Type II fibers) compared to the primarily slow-twitch soleus muscle (~80% Type I fibers) following heavy resistance exercise (231). A caveat when attempting to extrapolate such findings is that relatively high loads (>70% of 1RM) were used in a majority of studies on the topic, which potentially biases results in favor of fast-twitch fibers. Thus, it is conceivable that the superior capacity for hypertrophy of this particular fiber type may be a function of the models in which it has been studied rather than an inherent property of the fiber itself (158). The practical implications of this topic are discussed in later chapters.

Responses and Adaptations

Resistance exercise elicits a combination of neural and muscular responses and adaptations. Although an increased protein synthetic response is seen after a single bout of resistance training, changes in muscle size are not observed for several weeks of consistent exercise (207). Moreover, appreciable muscle protein accumulation (commonly referred to as *accretion*) generally takes a couple of months to become appreciably apparent (141). Early-phase increases in strength therefore are primarily attributed to neural improvements (141, 173, 196). Such observations follow the principles of motor learning. During the initial stages of training, the body is "getting used to" the movement patterns required for exercise performance. A general motor program must be created and then fine-tuned to carry out the exercise in a coordinated fashion. Ultimately, this results in a smoother, more efficient motor pattern and thus allows greater force to be exerted during the movement.

KEY POINT

Early-phase adaptations to resistance training are primarily related to neural improvements, including greater recruitment, rate coding, synchronization, and doublet firing.

Neural Drive

Several neural adaptations have been proposed to account for strength gains during acclimation to resistance training. Central to these adaptations is an increase in *neural drive*. Research indicates that humans are incapable of voluntarily producing maximal muscle force (55), but repeated exposure to resistance training enhances this ability. Numerous studies have reported increases in surface electromyography (EMG) amplitude after a period of regular resistance training, consistent with a heightened central drive to the trained muscles (2, 3, 80, 150). Research using the twitch interpolation technique, in which supramaximal stimuli are delivered to a muscle while subjects perform voluntary contractions, shows that as much as 5% of the quadriceps femoris muscle is not activated during maximal knee extension testing before exercise. After 6 weeks of training, however, subjects increased activation by an additional 2% (110). Similarly, Pucci and colleagues (174) reported an increase in voluntary activation from 96% to 98% after 3 weeks of training the quadriceps muscles. These results are consistent with research showing that trained athletes display greater muscle activation during high-intensity resistance exercise compared to nonathletes.

Muscle Activation

The findings of increased activation resultant to training are most often ascribed to a combination of greater *recruitment* (the number of fibers involved in a muscle action) and *rate coding* (the frequency at which the motor units are stimulated). It has been well established that muscle fiber recruitment follows the *size principle* (1, 12, 14, 16-19, 23, 33, 34). First explained by Henneman (90), the size principle dictates that the capacity of a motor unit to produce force is directly related to its size (figure 1.5). Accordingly, smaller, low-threshold, slow motor units are recruited initially during movement, followed by progressively larger, higher-threshold, fast motor units as the force demands increase for a given task. This orderly activation pattern allows for a smooth gradation of force, irrespective of the activity performed.

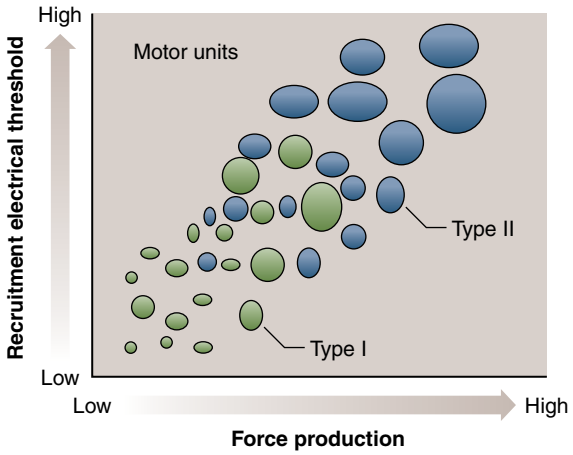

FIGURE 1.5 The Henneman size principle.

Two primary factors are responsible for the extent of muscle recruitment: level of muscle force and rate of force development. Training with heavy loads requires substantial force production and therefore calls on both low- and high-threshold motor units to maximize force. Although there is an intent to lift heavy loads quickly, the actual velocity of the lift is relatively slow. As the intensity of load decreases, the required force production from the muscle decreases, and fewer motor units are necessary to complete the lift given the same speed of shortening. By lifting a lighter weight quickly, however, most motor units are likely to be recruited even at loads equivalent to 33% of maximum (56). The extent of reductions in recruitment threshold from rapid contractions is greater for motor units in slow-contracting muscles, such as the soleus, compared with fast-contracting muscles, such as the masseter, one of the primary muscles involved in chewing food (56). The role of fatigue also must be considered with respect to recruitment. As fatigue increases during low-load contractions, the recruitment threshold of higher-threshold motor units progressively decreases even at somewhat slower speeds (95, 195, 242). It has been hypothesized that fatigue-induced reductions in motor unit threshold recruitment is an attempt by the neuromuscular system to sustain necessary levels of force generation to continue work output during repeated contractions (38).

The upper limit of motor unit recruitment is approximately 85% of maximal applied

isometric force; recruitment thresholds during dynamic actions are even lower (56). This suggests that enhancements in motor unit recruitment likely play a limited role in strength-related training adaptations. The ability to maximally recruit all available fibers in a given motor unit pool is essential for maximizing the hypertrophic response to resistance training. After all, the stimulus for a muscle fiber to adapt is predicated on its recruitment. However, it is important to note that simply recruiting a fiber does not necessarily promote a hypertrophic response. For example, a substantial recruitment of the full spectrum of muscle fibers, including those associated with high-threshold motor units, is achieved by cycling to fatigue at 75% of $\dot{V}O_2$max (195). Although this observation suggests that submaximal cycle exercise would promote substantial size increases across fiber types, research shows that muscle growth associated with aerobic exercise is limited primarily to Type I fibers (87).

Increases in force production above 85% of maximal voluntary contraction are thought to occur through greater discharge rates. Thus, an increase in rate coding would seem to be the most likely target for neural adaptation. Research is limited on the topic, but a study by Kamen and Knight (101) provides supporting evidence for training-induced enhancements in rate coding. Fifteen untrained young and older adults were tested for maximal voluntary contraction in knee extensions before and after 6 weeks of resistance exercise. By the end of the study, young subjects increased maximal discharge rate by 15%, and older subjects showed a 49% increase. Similarly, Van Cutsem and colleagues (234) showed that 12 weeks of resisted dorsiflexion training increased average firing frequency in the tibialis anterior from 69 to 96 pulses per second. In contrast, Pucci and colleagues (174) reported an increase of approximately 3% of maximal voluntary activation following 3 weeks of isometric quadriceps exercise, but no changes in discharge rate were noted. Differences in findings may be related to the methods employed for analysis. Recently, Del Vecchio and colleagues (51) demonstrated that changes in motor unit function of the tibialis anterior were mediated by adaptations in both recruitment and rate coding following 4 weeks of isometric strength training.

Motor Unit Synchronization

Several other factors have been speculated to account for neural improvements following resistance exercise. One of the most commonly hypothesized adaptations is an enhanced synchronization of motor units, whereby the discharge of action potentials by two or more motor units occurs simultaneously. A greater synchrony between motor units would necessarily result in a more forceful muscle contraction. Semmler and Nordstrom (204) demonstrated that motor unit synchronization varied when they compared skilled musicians (greatest degree of synchronization), Olympic weightlifters, and a group of controls (lowest degree of synchronization). However, other studies have failed to show increased synchronization following resistance training or computer simulation (105, 251). The findings cast doubt on whether synchronization plays a meaningful role in exercise-induced early-phase neuromuscular adaptations; if it does, the overall impact seems to be modest.

Antagonist Coactivation

Another possible explanation for exercise-induced neural enhancement is a decrease in antagonist coactivation. The attenuation of antagonist activity reduces opposition to the agonist, thereby allowing the agonist to produce greater force. Carolan and Cafarelli (41) reported that hamstring coactivation decreased by 20% after just 1 week of maximal voluntary isometric knee extension exercises, whereas no differences were seen in a group of controls. These findings are consistent with observations that skilled athletes display reduced coactivation of the semitendinosus muscle during open-chain knee extensions compared to sedentary people (13). The extent to which these adaptations confer positive effects on strength or hypertrophy remains unclear.

Doublets

An often-overlooked neural adaptation associated with resistance training is the effect on *doublets*, defined as the presence of two close

spikes less than 5 ms apart. Doublets often occur at the onset of contraction, conceivably to produce rapid force early on and thus generate sufficient momentum to complete the intended movement. Van Cutsem and colleagues (234) reported that the percentage of motor units firing doublets increased from 5.2% to 32.7% after 12 weeks of dynamic resisted dorsiflexion training against a load of 30% to 40% of 1RM. Interestingly, the presence of these doublets was noted not only in the initial phase of force development, but also later in the EMG burst. The findings suggest that doublet discharges contribute to enhancing the speed of voluntary muscle contraction following regimented resistance training.

Protein Balance

The maintenance of skeletal muscle tissue is predicated on the dynamic balance of muscle protein synthesis and protein breakdown. The human body is in a continual state of protein turnover; bodily proteins are constantly degraded and resynthesized throughout the course of each day. Skeletal muscle protein turnover in healthy recreationally active people averages approximately 1.2% a day and exists in dynamic equilibrium; muscle protein breakdown exceeds muscle protein synthesis in the fasted state and muscle protein synthesis exceeds muscle protein breakdown postprandially (19).

Protein synthesis has two basic components: transcription and translation (figure 1.6). Transcription occurs in the cell nucleus through a complex process that is segregated into three distinct phases: initiation, elongation, and termination. The process involves the creation of a *messenger ribonucleic acid* (mRNA) template that encodes the sequence of a specific protein

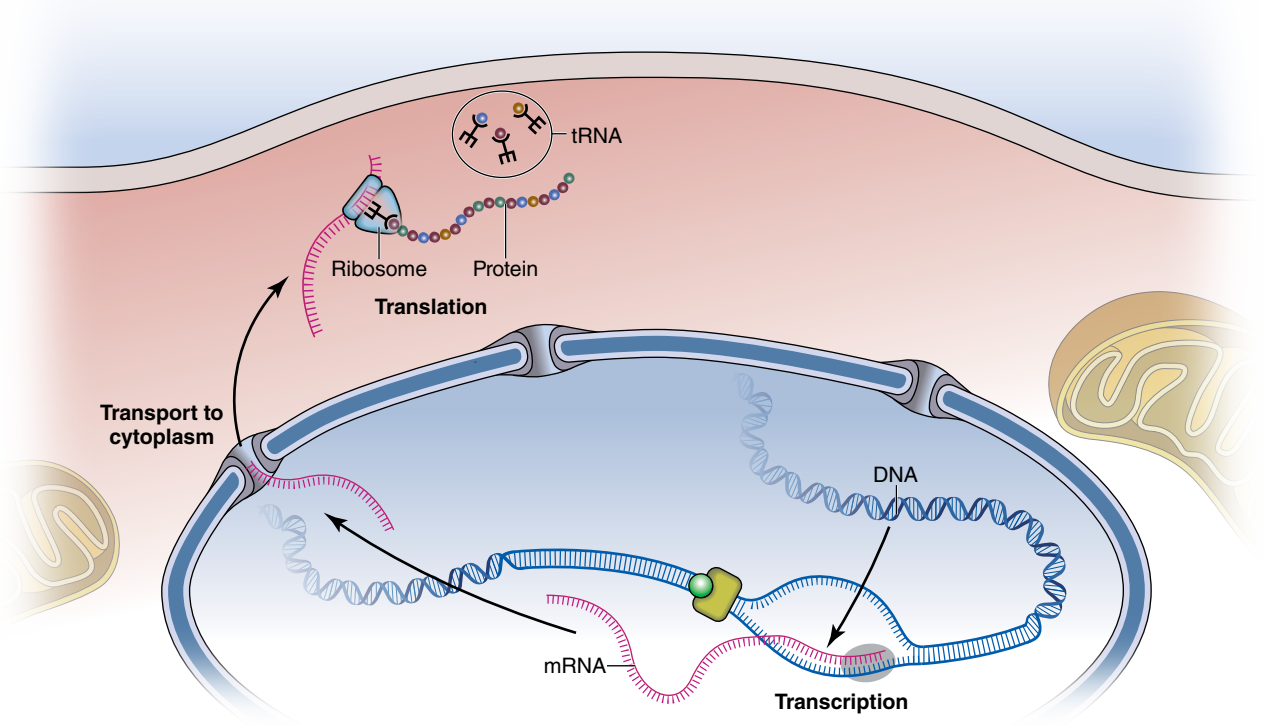

FIGURE 1.6 Protein translation and transcription—the basic processes of reading DNA sequence information and using it to build a protein molecule. The DNA sequence is read in the cell's nucleus, where a complementary RNA strand is built. That mRNA strand then moves to the cell cytoplasm, where it is used to manufacture the amino acid sequence of the protein.

from the genome. Each phase of transcription is regulated by various proteins (i.e., transcription factors, coactivators) that ensure the correct gene is transcribed in response to appropriate signals. Messenger ribonucleic acid concentration for a given protein is ultimately regulated by the myonuclear or the mitochondrial density and the transcription factors required for promoter activity (236).

Translation occurs in organelles called *ribosomes* located in the cell's sarcoplasm, which occupy approximately 20% of cell volume and comprise approximately 85% of total cellular RNA (64, 244). Ribosomes can be thought of as large peptide factories that regulate the translation of genetic material encoded in mRNA templates into muscle proteins. Each ribosome is composed of two subunits: a smaller subunit that binds the mRNA and a larger subunit that integrates specific transfer RNAs along with their bound amino acids (44). After binding with mRNA, the ribosomes synthesize a corresponding peptide strand by joining amino acids to *transfer ribonucleic acid* (tRNA) at the carboxyl end of the chain (44). The result is that translational capacity depends highly on the number of ribosomes in myocytes (5).

As with transcription, reactions are segregated into three phases: initiation, elongation, and termination. Each phase involves a distinct cluster of translation factors that are aptly termed *initiation factors* (eIF), *elongation factors* (eEF), and *release factors* (eRF) (the *e* stands for *eukaryotic,* referring to a cell that contains a nucleus and other cell structures). The availability and the state of activation of these factors determine the rate of translation of mRNA into muscle proteins (236). Translation initiation is believed to be the rate-limiting step in the protein synthetic response (130, 180). Not surprisingly, therefore, hormones and other growth factors that regulate muscle protein synthesis exert their effects by either increasing or decreasing the rate of translation initiation (44). That said, under certain circumstances, control of translation elongation can be critical to regulation of the protein synthetic rate (226).

During a bout of resistance training, muscle protein synthesis is suppressed and *proteolysis* (the breakdown of proteins into amino acids) is heightened so that net protein balance is in a net negative state. Note that protein breakdown resultant to exercise is considered an important component of exercise-induced hypertrophy because it helps to support amino acid reallocation as well as prevent the buildup of misfolded and nonfunctional proteins (133). After completion of the workout, muscle protein synthesis is increased 2- to 5-fold along with nutrient delivery, with effects lasting 48 hours or more post-exercise (168). An enhanced translational efficiency likely contributes to the exercise-induced increase in muscle protein synthesis (94, 160). Thus, when repeated bouts are performed over time and sufficient recovery is afforded between sessions, the synthetic response outpaces that of proteolysis, resulting in an increased accretion of muscle proteins.

Emerging evidence indicates that ribosome biogenesis is critical to increasing muscle mass. While translational efficiency appears to be a primary driver of the muscle protein synthesis response to exercise, the total number of ribosomes also plays an important role in the process (35, 244). The ribosomal pool is limited and must be expanded to support long-term growth because a given ribosome can translate only a finite amount of muscle proteins (183, 244). Numerous studies in both animals and humans have demonstrated strong correlations between muscle hypertrophy and ribosome biogenesis (244). Moreover, research in rodents shows that varying increases in hypertrophy following synergist ablation of 22%, 32%, and 45% are paralleled by dose-dependent increases in ribosomal content (1.8-fold, 2.2-fold, and 2.5-fold, respectively) (149); these findings emphasize the importance of expanding the number of ribosomes to realize progressively greater growth potential.

KEY POINT

Muscular adaptations are predicated on net protein balance over time. The process is mediated by intracellular anabolic and catabolic signaling cascades. Ribosome biogenesis is critical to maximizing hypertrophy over time.

Hypertrophy

By definition, muscle *hypertrophy* is an increase in the size of muscle tissue. During the hypertrophic process, contractile elements enlarge and the extracellular matrix expands to support growth (198). Growth occurs by adding sarcomeres, increasing noncontractile elements and sarcoplasmic fluid, and bolstering satellite cell activity.

Parallel and In-Series (Serial) Hypertrophy Contractile hypertrophy can occur by adding sarcomeres either in parallel or in series (figure 1.7). In the context of traditional exercise protocols, the majority of gains in muscle mass result from an increase of sarcomeres added in parallel (161, 224). Mechanical overload causes a disruption in the ultrastructure of the myofibers and the corresponding extracellular matrix that sets off an intracellular signaling cascade (see chapter 2 for a full explanation). With a favorable anabolic environment, this process ultimately leads to an increase in the size and amounts of the contractile and structural elements in the muscle as well as the number of sarcomeres in parallel. The upshot is an increase in the diameter of individual fibers and thus an increase in total muscle cross-sectional area (228).

Conversely, an in-series increase in sarcomeres results in a given muscle length corresponding to a shorter sarcomere length (228). An increase in serial hypertrophy has been observed in cases in which a muscle is forced to adapt to a new functional length. This occurs when limbs are placed in a cast and the corresponding immobilization of a joint at long muscle lengths leads to the addition of sarcomeres in series; immobilization at shorter lengths results in a reduction in sarcomeres (228). Cyclic stretch in rodent models also has shown to be a potent stimulator of in-series sarcomere addition (235).

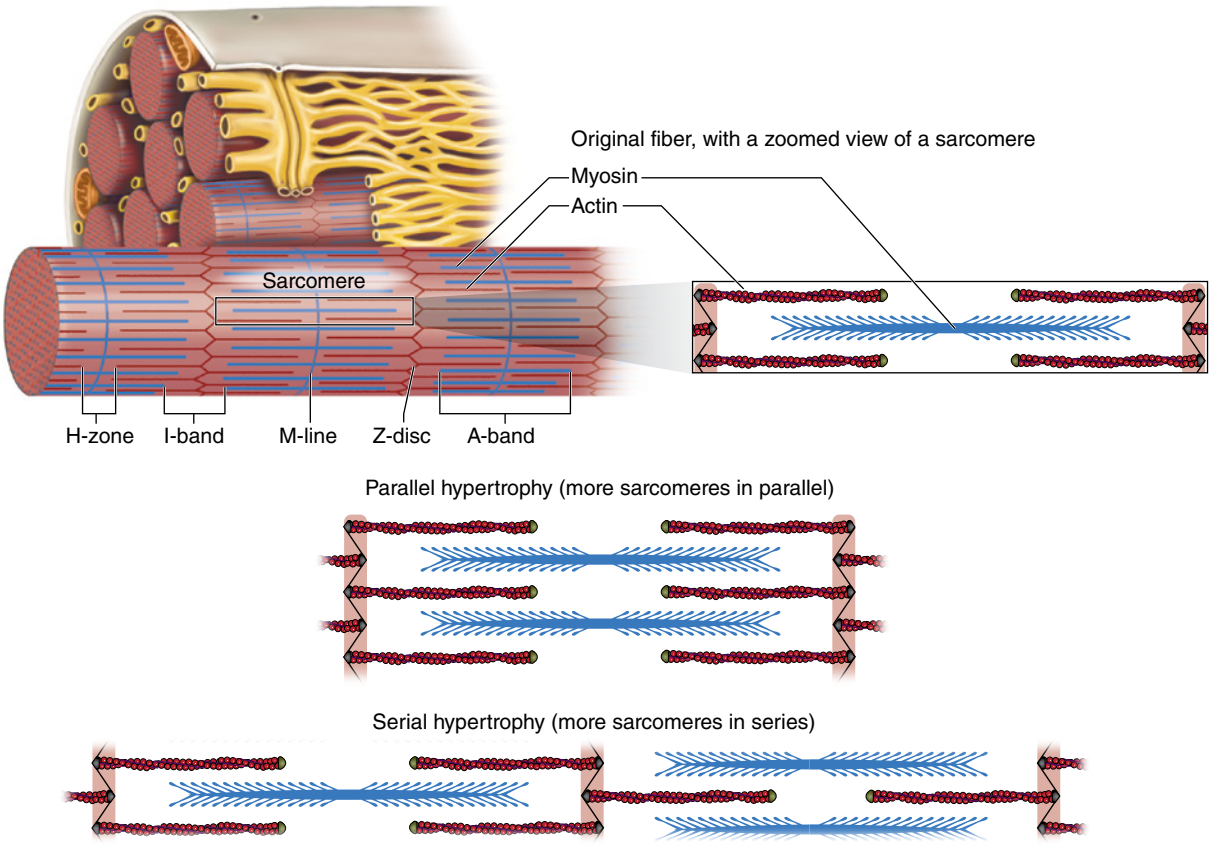

FIGURE 1.7 Parallel hypertrophy and serial hypertrophy.

KEY POINT

Hypertrophy can occur in series or in parallel. The primary means by which muscles increase in size following resistance training is through parallel hypertrophy.

Research indicates that certain types of exercise actions can affect fascicle length. There are three distinct types of actions: concentric, eccentric, and isometric. *Concentric actions* occur when a muscle is shortening; *eccentric actions* occur when a muscle is lengthening; and *isometric actions* occur when a muscle is producing force at an immobile joint. Lynn and Morgan (123) demonstrated lower sarcomere counts when rats climbed on a treadmill (i.e., incline) compared to when they descended (i.e., decline). This indicates that repeated eccentric-only actions result in a greater number of sarcomeres in series, whereas exercise consisting solely of concentric contractions leads to a serial decrease in sarcomere length, at least during unaccustomed aerobic-type exercise.

With respect to traditional resistance exercise, there is evidence that serial hypertrophy occurs to an extent during the early stages of participation. Seynnes and colleagues (207) reported a 9.9% increase in fascicle length in a group of recreationally active men and women after a 35-day high-intensity resistance training program. However, a longer-term study by Blazevich and colleagues (30) found that fascicle length changes were specific to the initial 5 weeks of resistance training, and that adaptations did not persist beyond this period. Evidence suggests that altering the style of training may influence changes in serial hypertrophy. Increases in fascicle length have been reported in athletes who replace heavy resistance training with high-speed training (11, 29). These findings suggest that performing concentric actions with maximal velocity may promote the addition of sarcomeres in series even in those with considerable training experience.

Sarcoplasmic Hypertrophy It is hypothesized that a training-induced increase in various noncontractile elements (i.e., collagen, organelles) and fluid may augment muscle size (126, 209). This phenomenon, often referred to as *sarcoplasmic hypertrophy*, conceivably enhances muscle bulk without concomitantly increasing strength (209). The sarcoplasmic component of muscle is illustrated in figure 1.8. Increases in sarcoplasmic hypertrophy are purported to be training specific—that is, lighter-load, higher repetitions promote greater accumulation of sarcoplasmic fractions compared to heavy-load, low repetitions. Support for this belief is based on research showing that muscle hypertrophy differs between bodybuilders and powerlifters (224). In particular, bodybuilders tend to display higher amounts of fibrous endomysial connective tissue as well as a greater glycogen content compared to powerlifters (125, 225), presumably as a result of differences in training methodology.

The chronic changes in intramuscular fluid are an intriguing area of discussion. Without question, exercise training can promote an increase in glycogen stores. MacDougall and colleagues (124) reported that resting concentrations of glycogen increased by 66% after 5 months of regimented resistance training. Moreover, bodybuilders display double the glycogen content of those who do not participate in regular exercise (4). Such alterations would

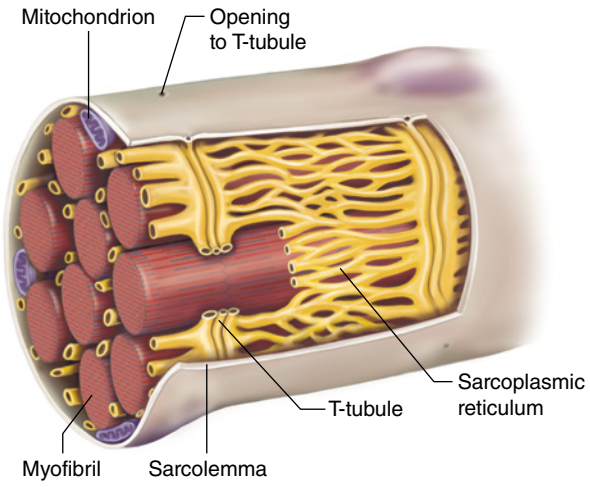

FIGURE 1.8 Sectional view of a muscle fiber showing the sarcoplasmic component of muscle.

seem to be mediated both by enzymatic alterations and the greater storage capacity of larger muscles. The relevance to sarcoplasmic changes is that 1 g of glycogen attracts approximately 3 to 4 g of water (42, 159).

Training-induced increases in intracellular hydration have been demonstrated after 16 weeks of progressive resistance training (185). Subjects performed a bodybuilding-type routine consisting of 3 sets of 8 to 12 repetitions with 60 to 90 seconds of rest between sets. A total of 11 exercises were performed per session using a combination of free weights, cables, and machines. All sets were taken to the point of momentary muscular failure. Analysis by bioelectrical impedance spectroscopy found significant increases in intracellular water content both at the midpoint of the study and at the study's end; results showed a moderate effect size. Conceivably, these alterations were mediated by increases in glycogen content because osmosis-promoting properties would be required to maintain the ratio of fluid to proteins and thus preserve the integrity of cellular signaling. Although the study provides evidence that training does in fact promote an increase in intracellular hydration (and, thereby, likely an increase in glycogen stores), what remains unclear is whether training-induced increases in intracellular hydration are specific to bodybuilding-type training or inherent to all types of resistance training. Bodybuilding-type training relies primarily on fast glycolysis to fuel performance, and glucose is the primary energy source. As such, the body necessarily adapts by increasing its capacity to store glycogen and thus fuel the demands of future performance. On the other hand, the short duration of powerlifting-type training requires that fuel be derived from immediately available ATP and PC sources. The lack of need to substantially use glucose during these bouts would seemingly diminish the need to ramp up glycogen storage capacity, and thus reduce localized fluid accumulation.

Although this line of reasoning provides a logical basis for training-specific alterations in sarcoplasmic volume, evidence that this occurs in practice remains equivocal. Burd and colleagues (37) found that training at 90% of 1RM induced greater early-phase post-exercise (~4 hours) increases in sarcoplasmic protein synthesis compared to training at 30% of 1RM, but the low-load condition showed a greater increase at 24 hours post-exercise. Although these findings are specific to myocellular protein fractions and do not necessarily reflect the long-term changes in hydration status associated with resistance training, the two are related. However, it is unknown whether such acute results would have persisted over time.

Recently, Haun and colleagues (89) provided intriguing longitudinal evidence that sarcoplasmic hypertrophy may in fact occur in the absence of myofibrillar growth in certain contexts. Thirty-one college-aged men with previous resistance training experience performed a regimented resistance training program that progressively increased volume from 10 sets per week to 32 sets per week over a 6-week training period. Fifteen subjects who exhibited notable muscle fiber cross-sectional area increases in the vastus lateralis, measured through muscle biopsy, were interrogated further to better understand the specific mode by which hypertrophy occurred. The results suggested that mitochondrial volumes decreased, glycogen concentrations were maintained, and, surprisingly, actin and myosin concentrations significantly decreased while sarcoplasmic protein concentrations tended to increase. From proteomic analyses, it appeared that proteins involved in anaerobic metabolism increased in expression. Collectively, the findings suggest that short-term, high-volume resistance training may elicit disproportionate increases in sarcoplasmic volume as opposed to hypertrophy of contractile elements. Given the limited current evidence on the topic, more research is warranted to provide confirmation or refutation of these results.

Satellite Cells Skeletal muscle is a postmitotic tissue, meaning that it does not undergo significant cell replacement throughout its life. An efficient means for regeneration of fibers is therefore required to maintain healthy

tissue and avoid cell death. It is widely accepted that satellite cells are essential to this process. These myogenic stem cells, which reside between the basal lamina and sarcolemma, remain inactive until a sufficient mechanical stimulus is imposed on skeletal muscle (239). Once aroused, they produce daughter cells that either self-renew to preserve the satellite cell pool or differentiate to become myoblasts that multiply and ultimately fuse to existing fibers, providing agents necessary for the repair and remodeling of the muscle (228, 254). This process is regulated by the Notch signaling pathway (208) and the transcription factor known as serum response factor (178). The satellite cell response may include the co-expression of myogenic regulatory factors such as Myf5, MyoD, myogenin, and MRF4 (47) that bind to sequence-specific DNA elements present in the promoter of muscle genes; each plays a distinct role in growth-related processes (193, 210). A subpopulation of satellite cells remains uninvolved in the adaptive mechanical response and instead is committed to self-renewal to ensure maintenance of the satellite cell pool (57).

The satellite cell response to a bout of resistance exercise lasts for many days, with effects peaking approximately 72 to 96 hours post-workout (23). The majority of evidence indicates that Type I fibers possess a greater resting number of satellite cells compared to Type II fibers, but it appears their population is increased to a greater extent in Type II fibers after resistance training (23). See figure 1.9.

It has been theorized that the most important hypertrophic role of satellite cells is their ability to retain a muscle's mitotic capacity by donating nuclei to existing myofibers (see figure 1.10), thereby increasing the muscle's capacity to synthesize new contractile proteins (22, 144). This phenomenon is generally considered obligatory for maximizing overload-induced hypertrophy (60).

Given that a muscle's ratio of nuclear content to fiber mass remains relatively constant

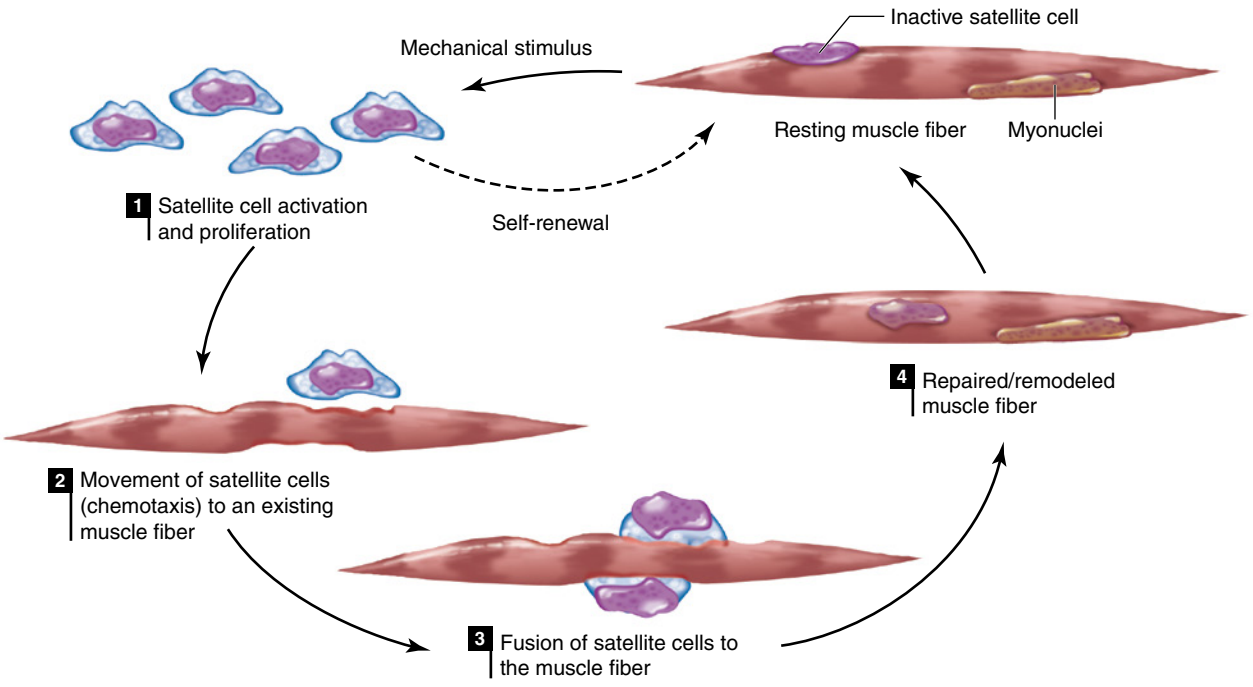

FIGURE 1.9 Cycle of satellite cell activation, differentiation, fusion, and repair and remodeling following a sufficient mechanical stimulus.

Adapted by permission from W.L. Kenney, J.H. Wilmore, and D.L. Costill, *Physiology of Sport and Exercise,* 6th ed. (Champaign, IL: Human Kinetics, 2015), 249.

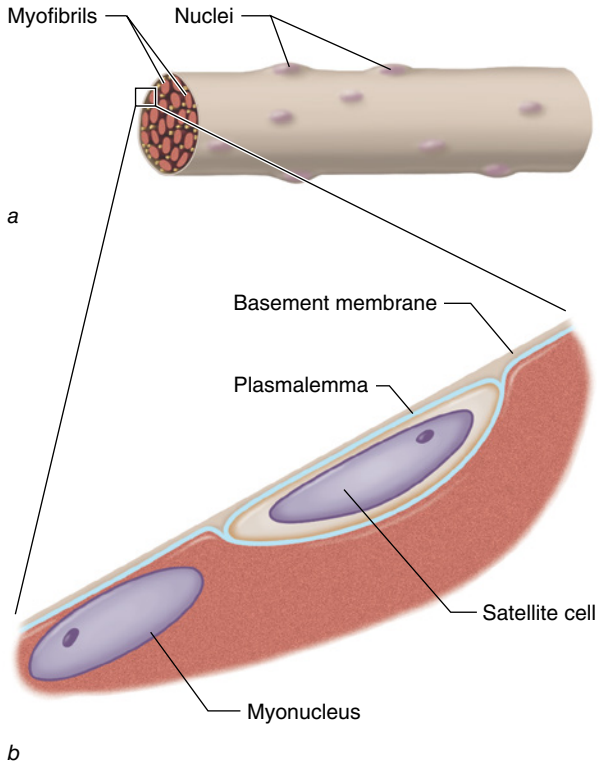

FIGURE 1.10 *(a)* Single muscle fiber with myonuclei at the periphery. *(b)* Myonucleus and satellite cell. The satellite cell is separated from the fiber by its own plasmalemma and that of the fiber, but it lies within the basement membrane of the skeletal muscle fiber.

during growth, the satellite cell–derived addition of myonuclei appears to be essential for sustaining muscular adaptations over the long term (227). This is consistent with the concept of *myonuclear domain*, which proposes that the myonucleus regulates mRNA production for a finite sarcoplasmic volume and any increases in fiber size must therefore be accompanied by a proportional increase in myonuclei (167). Considering that skeletal muscle contains multiple myonuclear domains, growth could occur by either an increase in the number of domains (via an increase in myonuclear number) or an increase in the size of existing domains. Both events are believed to occur during the adaptive response to exercise, and satellite cells are believed to contribute significantly to the process (228). Satellite cells may further contribute to increases in muscle size independent of myonuclear addition by regulating remodeling of extracellular matrix components (96).

KEY POINT

Satellite cells appear to be crucial to maximizing the hypertrophic response to resistance training. The primary role of satellite cells appears to be their ability to retain a muscle's mitotic capacity by donating nuclei to existing myofibers, and they may contribute to hypertrophic gains in other ways as well.

Although controversy exists regarding the precise hypertrophic role of satellite cells (132), the prevailing body of research indicates that they are crucial for the regulation of load-induced muscular growth (6, 157). Compelling support for this contention was demonstrated in a cluster analysis by Petrella and colleagues (167) that showed extreme hypertrophic responders to resistance training (>50% increases in mean myofiber cross-sectional area of the vastus lateralis over the course of a 16-week study period) displayed a much greater capacity to expand the satellite cell pool compared to those who experienced moderate or negligible increases in growth. More recently, Bellamy and colleagues (24) showed a strong positive relationship between the acute temporal satellite cell response to 16 weeks of resistance training and subsequent muscle protein accretion. Correlations were noted in all fiber types, and expansion of the satellite cell pool showed the greatest associated hypertrophic increases in Type II fibers. Satellite cells also play an essential role in regulation of the extracellular matrix, which has been shown to be integrally involved in mediating exercise-induced hypertrophic adaptations (67, 146) and replenishment of the satellite cell pool (181). These findings are consistent with research showing that hypertrophy is significantly impaired when satellite cells are obliterated by gamma irradiation (238).

It seems likely that satellite cells become relevant only when muscle growth reaches a certain threshold. Kadi and colleagues (100) found that increases in myofiber hypertrophy of up to 15% could be achieved without significantly adding new myonuclei; however, myonuclear addition was required when hyper-

trophy reached 26%, conceivably because of an inability to further expand the myonuclear domain. This observation suggests that satellite cell function might be particularly important in well-trained people because the size of myofibers would necessarily reach the upper limits of their myonuclear domain. Despite speculation that the threshold for myonuclear addition occurs when increases in myofiber size reach approximately 26%, this does not necessarily play out in practice (146). Thus, rather than a "rigid" myonuclear domain, the threshold at which nuclei are required to sustain fiber growth appears to be flexible (146).

Interestingly, myonuclei appear to be maintained over time even after long periods of detraining and the corresponding muscle atrophy. In animal models, a technique called *synergist ablation* is often used to study muscle tissue; the process involves an agonist muscle being surgically removed so that other synergist muscles are forced to carry out a movement (see chapter 4). In an elegant design, Bruusgaard and colleagues (36) used synergist ablation to cause significant hypertrophy in the extensor digitorum muscle of rodents and a 37% increase in myonuclei count. Subsequent denervation of a parallel group of animals produced marked muscular atrophy, but the number of myonuclei remained constant (36). Work from the same lab showed that mice treated with testosterone propionate for 14 days elicited a 77% increase in muscle hypertrophy and a 66% increase in myonuclei count (59). Muscle fiber size returned to baseline levels 3 weeks after discontinuation of steroid administration. However, the myonuclei count remained elevated for at least 3 months, which amounts to over 10% of the animal's life span. These findings indicate that the retention of satellite cells associated with hypertrophic adaptations serves as a cellular memory mechanism that helps to preserve the future anabolic potential of skeletal muscle (59), although a recent study found that satellite cell accretion in mice subjected to 8 weeks of resistive exercise returned to basal levels following 12 weeks of detraining (58). Based on the preponderance of current research, the number of myonuclei might be limited by a person's ability to add muscle during the initial stages of overload, but the subsequent addition of satellite cell–derived nuclei associated with muscle protein accretion might facilitate increased synthesis upon retraining (77, 202).

Hyperplasia

It has been theorized that exercise-induced muscle growth may be due in part to *hyperplasia*—an increase in fiber number (figure 1.11). Evidence supporting the ability of muscles to undergo hyperplasia is primarily derived from animal research. Alway and colleagues (12) attached a weight to the right wings of adult Japanese quails that corresponded to 10% of their body mass. The contralateral limb served as a control. After 5 to 7 days of chronic stretch, fiber number was approximately 27% greater than that in nonloaded controls. These findings indicate a substantial contribution of hyperplasia to gains in lean mass. Follow-up work by the same lab evaluated a comparable stretch protocol except that loading was carried out for 24-hour intervals interspersed with 48- to 72-hour rest periods (16). Although significant increases in mean cross-sectional fiber area were noted in the stretched limb, fiber number did not change over the course of the study. Subsequent work by the lab expanded on this study to employ progressive overload (17). Loading was increased from 10% to 35% of the bird's body mass over a period of 28 days, interspersed by short periods of unloading. Histological analysis determined an 82% increase in fiber number at the study's end. These findings seem to indicate that extreme loading conditions can induce hyperplasia, at least in an avian model. It is hypothesized that once fibers reach a critical size threshold, they cannot enlarge further and thus split to allow additional hypertrophy to occur.

Whether hyperplasia occurs in humans using traditional training protocols remains controversial. A meta-analysis on the topic of 17 studies meeting inclusion criteria concluded that a stretch overload consistently produced greater fiber counts, and exercise-based protocols produced highly inconsistent results (103). Moreover, increases in myofiber number were substantially greater in studies that used avian

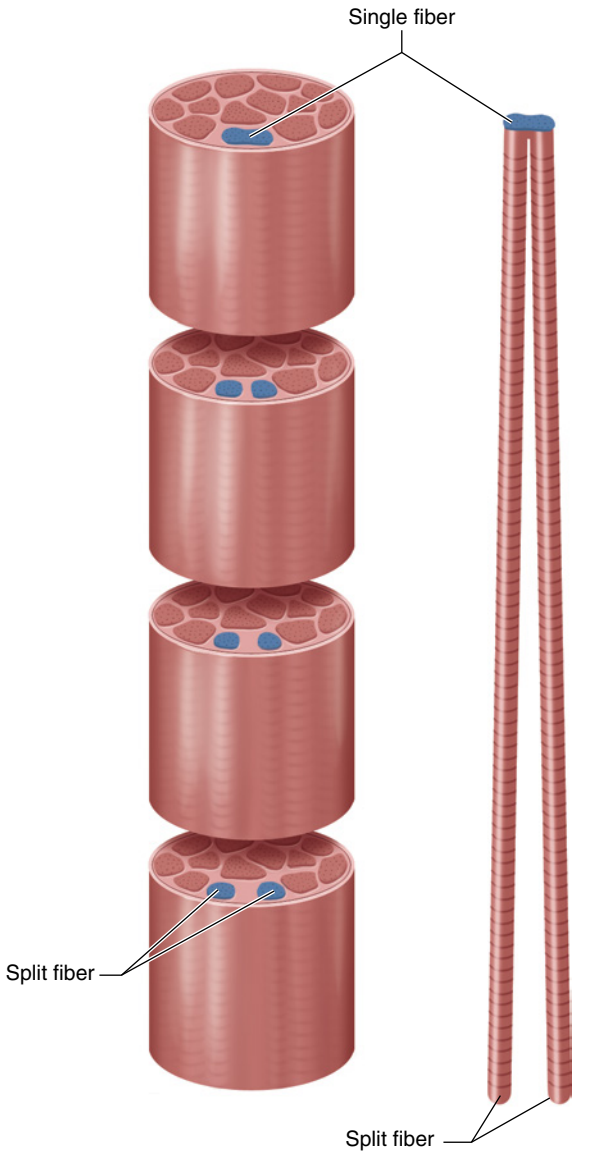

FIGURE 1.11 Muscle fiber splitting (hyperplasia).

(~21%) versus mammalian (~8%) models. MacDougall and colleagues (126) evaluated myofiber count of the biceps brachii in 5 elite male bodybuilders, 7 intermediate-caliber bodybuilders, and 13 age-matched controls. Despite markedly greater hypertrophy in the bodybuilders, the fiber counts of the groups were similar, indicating that consistent intense resistance training had no effect on hyperplasia. Paul and Rosenthal (161) proposed that the authors of studies showing evidence of hyperplasia may have misinterpreted the intricate arrangements of elongating fibers as increases in fiber number. These researchers noted the difficulty in attempting to analyze fiber count, particularly in pennated muscles in which fibers do not all lie in the plane of sectioning, and in muscles with multiple endplate bands and many intrafascicularly terminating fibers in series. The body of evidence suggests that the notion that new myofiber formation contributes to loading-induced muscle hypertrophy in humans is questionable. If a contribution does exist, its impact on increases in muscle cross-sectional area appears to be minimal (6). Most likely, humans cannot naturally increase muscle size to reach the critical threshold that warrants fiber splitting. It remains possible that administration of supraphysiological doses of illicit anabolic agents may result in extreme hypertrophy that allows individuals to exceed the limits of hypertrophic capacity and thus promotes hyperplasia (147).

Endocrine, Paracrine, and Autocrine Systems

Muscle protein balance is influenced, in part, by the neuroendocrine system. Various hormones have been shown to alter the dynamic balance between anabolic and catabolic stimuli in muscle, helping to mediate an increase or decrease in muscle protein accretion (212). Moreover, certain substances (hormones and myokines) are secreted locally, either in a *paracrine* (between adjacent cells) or *autocrine* (within the cell itself) fashion, in response to exercise to cause specific adaptations.

Responses and Adaptations of Hormones

Endocrine hormones are produced within glands, released into the blood, and then transported to target tissues where they bind to receptors either on the sarcolemma or in the sarcoplasm. Table 1.2 provides a summary of the primary anabolic hormones and their actions. There is clear and compelling evidence that basal concentrations of anabolic hormones influence growth and regenerative capacity of skeletal muscle (46); when anabolic hormonal

TABLE 1.2 Primary Hormones and Their Respective Actions

Hormone	Actions
Insulin-like growth factor-1 (IGF-1)	Primary hypertrophic effects of the systemic isoform appear to be in stimulating differentiation and fusion following myotrauma and thereby facilitating the donation of myonuclei to muscle fibers. Although IGF-1 does directly influence anabolic intracellular signaling, it is not clear whether these effects are synergistic for exercise-induced muscle growth.
Growth hormone (GH)	Anabolic effects of GH on muscle tissue are carried out primarily via its potentiating effect on IGF-1. Although some evidence supports that GH promotes anabolism independent of IGF-1, it remains questionable whether these effects have an appreciable impact on postnatal muscle development.
Testosterone	Directly increases myofibrillar protein synthesis and decreases proteolysis. Potentiates the release of GH and IGF-1 while inhibiting activity of IGFBP-4. Increases the number of myogenically committed satellite cells.
Insulin	Primary effect on exercise-induced hypertrophic adaptations is believed to be a reduction in protein breakdown as opposed to increases in MPS.

concentrations are chronically suppressed, muscular adaptations are blunted. The following sections address the hypertrophic role of the primary anabolic hormones (insulin-like growth factor 1, growth hormone, testosterone, and insulin) and the resistance training–mediated alterations caused by these hormones.

Insulin-Like Growth Factor 1

Insulin-like growth factor 1 (IGF-1) is a homologous peptide that, as the name implies, has structural similarities to insulin. IGF-1 carries out intracellular signaling via multiple pathways (see chapter 2) (78, 189, 205). These signaling cascades have both anabolic and anticatabolic effects on muscle and thus promote increased tissue growth (197). *In vitro* research (studies done in a laboratory setting on extracted cells, not inside the body) consistently shows that IGF-1 incites protein synthesis, inhibits protein breakdown, and increases both myotube diameter and the number of nuclei per myotube (88). Despite its known anabolic properties, however, evidence suggests that a functional IGF-1 receptor is not essential for exercise-induced muscle hypertrophy (214).

Three distinct IGF-1 isoforms have been identified in humans: IGF-1Ea, IGF-1Eb, and IGF-1Ec. Both IGF-1Ea and IGF-1Eb are produced mainly in the liver and then released into systemic circulation. Other tissues express these isoforms as well, however, and the extent of nonhepatic synthesis increases in response to physical activity. In fact, contracting muscles produce the majority of systemic IGF-1 during intense exercise, and much of the circulating IGF-1 is inevitably taken up by active myofibers (33, 71). On the other hand, IGF-1Ec is a splice variant of the IGF-1 gene specific to muscle tissue. It is expressed in response to mechanical loading and then carries out its actions in an autocrine/paracrine fashion (71). Because IGF-1Ec is stimulated mechanically, and given that its carboxy peptide sequence is different from the systemic isoform, it has been termed *mechano growth factor* (MGF). (Because MGF carries out its actions locally as opposed to systemically, it is specifically discussed in the section on myokines and only briefly covered in this section.)

The age-related decrease in serum IGF-1 levels is associated with muscle atrophy (84); this suggests that a minimum threshold exists for circulating concentrations of this hormone, below which muscle mass is compromised. IGF-1 is a potent effector of the PI3K/Akt pathway (see chapter 2) and is widely thought to be necessary for activating the signal transduction required for the initiation of protein translation following mechanical loading (215). However, the extent to which systemic IGF-1 is involved in compensatory hypertrophy remains controversial, and some researchers dispute whether it has a primary role in the anabolic response

to exercise (132, 157). Serum concentrations of IGF-1 are not necessarily correlated with post-workout increases in muscle protein synthesis (257). Furthermore, IGF-1–deficient mice exhibiting an 80% reduction in circulating IGF-1 levels do not exhibit an impaired hypertrophic response to resistive exercise (128). The inconsistencies in studies on this topic have yet to be reconciled.

The upregulation of systemic IGF-1 is delayed following exercise, and this temporal pattern of release coincides with later-stage satellite cell regulation (166). Hence, the primary hypertrophic effects of systemic IGF-1 may manifest in its ability to stimulate differentiation and fusion following myotrauma and thereby facilitate the donation of myonuclei to muscle fibers to maintain optimal DNA-to-protein ratios (228, 238). Whether the systemic IGF-1 isoforms have additional hypertrophic actions as a result of resistance training remains to be established.

Growth Hormone

Growth hormone (GH) is a superfamily of polypeptide hormones released by the anterior pituitary gland. GH is secreted in a pulsatile manner, and the highest nonexercise emission takes place during sleep. GH possesses both anabolic and catabolic properties (238). On one hand, it stimulates *lipolysis* (the breakdown of lipids); on the other hand, it promotes cellular uptake and the incorporation of amino acids into various proteins (239). Although there is evidence that endogenous GH plays a role in the regulation of skeletal muscle mass (238), at physiological levels its primary anabolic action appears to be more specific to collagen synthesis as opposed to increasing accretion of myofibrillar proteins (54).

The anabolic influence of GH on muscle tissue is thought to be carried out primarily via its potentiative effect on IGF-1 (238). Animal research shows that an increase in skeletal muscle mass associated with GH requires an intact IGF-1 receptor (106). These findings are consistent with studies showing significant increases in circulating IGF-1 levels following GH administration (18, 83, 188). In addition to mediating the release of systemic IGF-1 isoforms, GH also appears to increase the action of MGF. Klover and Hennighausen (109) found that removing the genes for signal transducers and activators of transcription (STAT), which are considered compulsory regulators of GH-induced transcription of the IGF-1 gene, led to a selective loss of skeletal muscle STAT5 protein, whereas hepatic expression remained unaltered (109). These findings are consistent with in vitro research showing that treating myoblast C2C12 cells with recombinant GH directly potentiates MGF expression before that of IGF-1Ea (99). In addition, the administration of GH in mice significantly elevated MGF, indicating that MGF mRNA expression occurs in parallel with GH release (98). Alternatively, GH-independent expression of IGF-1Ea and MGF has been observed in hypophysectomized (pituitary gland removed) rats following synergist ablation (249), which implies that GH serves to potentiate rather than regulate IGF-1 function. Interestingly, there is evidence that mRNA levels of MGF are greatly increased when elderly men combine resistance training with recombinant GH treatment (83), but similar results are not seen in young adult men (18). Discrepancies in findings are not clear, but there may be a minimum level of GH required to mediate MGF production. It is conceivable that age-related reductions in the hormone may lead to a deficiency that requires exogenous GH administration to reach the required threshold.

The claim that GH mediates hypertrophy solely via potentiating IGF-1 release remains controversial. Some researchers have suggested that the two hormones may confer additive effects (213, 238). The possibility of IGF-1–independent anabolic effects of GH is indicated by research showing reduced growth retardation in IGF-1 knockout mice compared to those lacking both an IGF-1 and GH receptor (122). Moreover, a reduction in myofiber size is seen in skeletal muscle deficient of functional GH receptors (213). These effects are thought to be carried out, at least in part, by later-stage GH-regulated cell fusion that results in an increase in the number of nuclei per myotube (213). The actions of GH also seem to cause a permissive, or perhaps even a synergistic, effect on testosterone-mediated

muscle protein synthesis (240). Whether these effects are seen as a result of endogenous GH production within normal physiological levels remains speculative.

Testosterone

Testosterone is a steroidal hormone derived from cholesterol in the Leydig cells of the testes via the hypothalamic-pituitary-gonadal axis, and small quantities are synthesized in the adrenals and ovaries (39). Men have an amount of circulating testosterone approximately 10-fold greater than women, and this hormonal discrepancy between the sexes is believed to be in large part responsible for the greater muscularity seen in postpubescent males (88). The overwhelming majority of circulating testosterone is bound to either sex hormone–binding globulin (60%) or albumin (38%); the residual amount of approximately 2% circulates in an unbound state. Unbound testosterone is biologically active and available to be taken up by bodily tissues; weakly bound testosterone can rapidly dissociate from albumin and become active (119). In its unbound form, testosterone binds to androgen receptors in the cytoplasm of target tissues. This causes a conformational change that shuttles the testosterone–androgen receptor complex to the nucleus of the cell, where it regulates gene transcription (240).

The anabolic actions of testosterone are irrefutable. The administration of exogenous testosterone produces large increases in muscle mass in both men and women regardless of age (25, 27, 210), and these effects are amplified when combined with resistance training (26). Elderly women display significantly greater exercise-induced growth when testosterone concentrations are chronically high versus low (81, 82). Kvorning and colleagues (116) showed that blunting testosterone production in young men by administering goserelin, a gonadotropin-releasing hormone analogue, significantly impaired muscular adaptations after 8 weeks of resistance training.

The anabolic actions of testosterone have been partly attributed to its direct ability to increase protein synthesis and diminish proteolysis (233, 256). It also is suggested that testosterone increases the release of other anabolic agents, including GH (237) and IGF-1/MGF (203), while inhibiting the activity of IGFBP-4, which is an IGF-1 antagonist (233). Evidence also shows that the combined elevation of testosterone and GH acts synergistically to increase IGF-1 (240). Moreover, myoblasts have been shown to contain androgen receptors. Accordingly, evidence suggests a dose-dependent effect of testosterone on satellite cell proliferation and differentiation, and that higher testosterone concentrations increase the number of myogenically committed cells (88, 210). Detrimental effects of low testosterone levels on muscle mass appear to be more related to an accelerated rate of proteolysis than to an attenuation of muscle protein synthesis (191).

The normal range for total testosterone levels in healthy young men is 264 to 916 ng/dL (232). Although research shows that hypogonadism (defined as a testosterone level 2 standard deviations below the mean for healthy young men) results in an impaired ability to build muscle (32, 116), it is not clear whether testosterone fluctuations within the normal physiological range affect hypertrophy. Some research indicates that disparate effects are seen at the extremes of the range, with those in the upper range showing more favorable measures of lean mass than those in the lower range (145). However, evidence remains indeterminate as to whether muscle-building differences exist in the midrange of normal values (i.e., approximately 400 to 700 ng/dL). Although some studies show that long-term adherence to regimented resistance training can increase basal testosterone levels, these findings are not universal (93).

There is evidence that the quantity of androgen receptors may play a role in the anabolic response to exercise (10). Androgen receptor concentration is diminished immediately after resistance training, but levels rise significantly over the ensuing several hours (240). Indeed, evidence suggests an association between post-exercise androgen receptor content and muscle hypertrophy (142). Some studies indicate this post-exercise androgen receptor upregulation is dependent on corresponding elevations in testosterone levels (216), while others do not support such a relationship (142).

The immediate acute rise in testosterone levels post-exercise followed by the combination of its rapid decline (within ~1 hour) and corresponding upregulation of the muscle androgen receptor may suggest a movement of testosterone from circulation into the muscle tissue (93).

Overall, the findings on whether acute testosterone spikes influence exercise-induced hypertrophic adaptations either directly or through their effects on androgen receptors are conflicting; more importantly, the practical relevance of such an effect, if it does in fact occur, remains questionable (see the discussion on acute versus chronic hormonal responses later in the chapter).

Insulin

Insulin is a peptide hormone secreted by the beta cells of the pancreas. In healthy people, insulin regulates glucose metabolism by facilitating its storage as glycogen in muscle and liver tissue. Among other secondary roles, insulin is involved in muscle anabolism, stimulating both the initiation and elongation phases of protein translation by regulating various eIFs and eEFs. Insulin also exerts anabolic effects through activation of the *mammalian target of rapamycin*, universally abbreviated as *mTOR*. A serine/threonine protein kinase, mTOR plays a critical role in regulating cell growth and monitoring cellular nutrient, oxygen, and energy levels (see the PI3K/Akt pathway discussion in chapter 2 for more information).

Despite its anabolic properties (28, 65), the primary role of insulin on exercise-induced hypertrophic adaptations is believed to be a reduction in protein breakdown as opposed to promoting increases in muscle protein synthesis (52, 69, 91, 104). The mechanisms by which insulin reduces proteolysis are not well understood at this time. Given that muscle hypertrophy represents the difference between muscle protein synthesis and proteolysis, a decrease in protein breakdown would conceivably enhance the accretion of contractile proteins and thus facilitate greater hypertrophy.

It should be noted that in nondiabetic populations, exercise has little effect on insulin levels and can actually blunt its release depending on intensity, duration, and pre-exercise nutritional consumption (115). Rather, the primary mechanism to manipulate insulin is through nutrient provision. Thus, its hypertrophic role is further explored in chapter 9 in the discussion of nutrient timing strategies.

Acute Versus Chronic Hormonal Responses

Exercise has been shown to significantly increase the release of anabolic hormones in the immediate post-workout period. Strong correlations have been shown between hypertrophy-type training and acute hypophyseal GH secretion (74-76, 79, 170, 219, 220), and the magnitude of these increases is sizable. Fujita and colleagues (68) reported a 10-fold increase in GH levels following blood flow restriction exercise (see chapter 2), whereas Takarada and colleagues (220) found that elevations reached 290-fold over baseline. It is believed that elevations are at least in part mediated by metabolite production (74, 79). An increase in acidosis from H+ buildup also may potentiate GH production via chemoreflex stimulation regulated by intramuscular metaboreceptors and group III and IV afferents (120, 241).

Performance of hypertrophy-type training also has been shown to significantly increase circulating IGF-1 levels (112, 113, 192), although these results have not been consistent across all trials (114). It is not clear whether such elevations are mediated primarily by corresponding increases in GH release or whether the exercise itself enhances acute production. Research on the acute testosterone response to resistance training has been somewhat inconsistent. Several studies have shown greater elevations in testosterone following hypertrophy-type resistance training versus strength-type protocols (39, 76, 79, 134, 211), whereas others failed to detect significant differences (112, 182, 218). It should be noted that sex, age, and training status profoundly influence testosterone synthesis (115), and these factors may account for conflicting results.

Given the positive relationship between anabolic hormones and hypertrophy-type training, researchers formulated the *hormone hypothesis*, which proposes that post-workout hormonal elevations are central to long-term increases

in muscle size (75, 85). It has been proposed that these momentary hormonal spikes may be more important to muscle growth–related responses than chronic alterations in resting hormonal concentrations (115). Theoretically, hormonal spikes increase the likelihood that the secreted hormones interact with target tissue receptors (48), which may be especially beneficial after exercise when muscles are primed for tissue anabolism. In addition, large hormonal elevations may positively influence intracellular signaling to rapidly reduce post-exercise proteolysis and heighten anabolic processes to achieve a greater supercompensatory response.

Despite a seemingly logical basis, a number of researchers have questioned the legitimacy of the hormone hypothesis (121, 169) and have proposed an alternative hypothesis that such biological events are intended to mobilize fuel stores rather than promote tissue anabolism (247). In particular, the anabolic role of acute GH production has been dismissed largely based on studies showing that injections of genetically engineered recombinant GH do not promote greater increases in muscle growth (118, 252, 253). Although this contention may have merit, it fails to take into account the fact that exogenous GH administration does not mimic the *in vivo* (within a whole, living organism) response to exercise-induced hormonal elevations either temporally or in magnitude. The intracellular environment is primed for anabolism following intense training, and it is conceivable that large transient spikes in GH enhance the remodeling process. Moreover, recombinant GH is composed solely of the 22-kDa isoform (61), whereas more than 100 molecular isoforms of GH are produced endogenously (154). These isoforms peak in the early post-exercise period, and a majority of those isoforms are of the non-22-kDa variety (61). Recombinant GH administered in supraphysiological doses (i.e., a dose that is larger or more potent than would occur naturally in the body) actually inhibits the post-workout stimulation of these alternative isoforms (61), and thus conceivably could blunt anabolism. Whether these factors significantly affect hypertrophic adaptations has yet to be established.

The binding of testosterone to cell receptors can rapidly (within seconds) trigger second messengers involved in downstream protein kinase signaling (49), suggesting a link between momentary post-workout elevations and muscle protein synthesis. Kvorning and colleagues (117) demonstrated that suppressing testosterone levels with goserelin blunted exercise-induced muscle growth despite no alterations in acute mRNA expression of MyoD, myogenin, myostatin, IGF-1Ea, IGF-1Eb, IGF-1Ec, and androgen receptor, suggesting that testosterone may mediate intracellular signaling downstream from these factors. Both total and free testosterone levels in the placebo group increased by approximately 15% immediately post-exercise, whereas those treated with goserelin displayed a reduction in total and free testosterone 15 minutes after the training bout, suggesting an anabolic effect from the transient elevations. In contrast to these findings, West and colleagues (245) reported that acute elevations in post-exercise anabolic hormones had no effect on post-exercise muscle protein synthesis in young men compared to those performing a protocol that did not significantly elevate hormones. Although these studies provide insight into general hypertrophic responses, it is important to recognize that the acute protein synthetic response to exercise training does not always correlate with chronic anabolic signaling (45), and these events are not necessarily predictive of long-term increases in muscle growth (227). This is particularly true with respect to the untrained subjects used in these studies because their acute responses may be more related to their unfamiliarity with the exercise per se and the associated muscle damage that inevitably occurs from such training (19).

Several longitudinal studies show significant associations between the post-exercise hormonal response and muscle growth. McCall and colleagues (131) investigated the topic in 11 resistance-trained young men over the course of a 12-week high-volume resistance training program. Strong correlations were found between acute GH increases and

the extent of both Type I (r = .74) and Type II (r = .71) fiber cross-sectional area. Similarly, Ahtiainen and colleagues (9) demonstrated strong associations between acute testosterone elevations and increases in quadriceps femoris muscle cross-sectional area (r = .76) in 16 young men (8 strength athletes and 8 physically active people) who performed heavy resistance exercise for 21 weeks. Both of these studies were limited by small sample sizes, compromising statistical power. Subsequently, several larger studies from McMaster University cast doubt on the veracity of these findings. West and Phillips (248) studied the post-exercise systemic response to 12 weeks of resistance training in 56 untrained young men. A weak correlation was found between transient GH elevations and increases in Type II fiber area (r = .28), which was estimated to explain approximately 8% of the variance in muscle protein accretion. No association was demonstrated between the post-exercise testosterone response and muscle growth. Interestingly, a subanalysis of hormonal variations between hypertrophic responders and nonresponders (i.e., those in the top and bottom ~16%) showed a strong trend for correlations between increased IGF-1 levels and muscular adaptations (p = .053). Follow-up work by the same lab found no relationship between acute elevations in testosterone, GH, or IGF-1 and mean increases in muscle fiber cross-sectional area following 16 weeks of resistance training in a group of 23 untrained young men (140). Although the aforementioned studies provide insight into possible interactions, caution must be used in attempting to draw causal conclusions from correlative data.

A number of studies have attempted to directly evaluate the effect of the transient post-exercise hormonal release on muscle protein accretion. The results of these trials have been conflicting. Madarame and colleagues (127) found a significant increase in elbow flexor cross-sectional area following unilateral upper-arm exercise combined with lower-body occlusion training compared to identical arm training combined with non-occluded lower-body exercise. Differences in GH levels between conditions did not rise to statistical significance, but the authors stated that this was likely a Type II error due to lack of statistical power. Given that comparable protocols have resulted in marked increases in post-exercise hormones (74, 75, 79, 170, 219, 220), findings suggest a possible role of systemic factors in the adaptive response. It also should be noted that muscle cross-sectional area remained unchanged in the nontrained arm, indicating that the acute systemic response had no hypertrophic effect in the absence of mechanical stimuli.

Employing a within-subject design, West and colleagues (246) recruited 12 untrained men to perform elbow flexion exercise on separate days under two hormonal conditions: a low-hormone condition in which one arm performed elbow flexion exercise only and a high-hormone condition in which the contralateral arm performed the same arm curl exercise followed immediately by multiple sets of lower-body resistance training designed to promote a robust systemic response. After 15 weeks, increases in muscle cross-sectional area were similar between conditions despite significantly higher post-exercise concentrations of circulating IGF-1, GH, and testosterone in those in the high-hormone condition.

Ronnestad and colleagues (190) carried out a similar within-subject design as that of West and colleagues (246), except that the high-hormone group performed lower-body exercise before elbow flexion exercise. In contrast to the findings of West and colleagues (246), significantly greater increases in elbow flexor cross-sectional area were noted in the high-hormone condition, implying a causal link between acute hormonal elevations and hypertrophic adaptations. Differences were region specific, and increases in cross-sectional area were seen only at the two middle sections of the elbow flexors where muscle girth was largest.

Most recently, Morton and colleagues (142) reported that increases in hypertrophy pursuant to a 12-week total-body strength training program were unrelated to acute hormonal elevations. Importantly, this study employed a cohort of 49 resistance-trained men, indicating that previous resistance training experience does

KEY POINT

The endocrine system is intricately involved in the regulation of muscle mass, although the exact role of acute hormonal elevations in hypertrophy is unclear and likely of minor consequence. The chronic production of testosterone, growth hormone, IGF-1, and other anabolic hormones influences protein balance to bring about changes in resistance training–mediated muscular adaptations.

not factor into the relevance of post-exercise systemic responses to muscular adaptations.

Evidence from the body of literature as to whether post-exercise anabolic hormonal elevations are associated with increases in muscle growth remains murky. Although it is premature to completely dismiss a potential role, it seems likely that if such a role does exist, the overall magnitude of the effect is at best modest (199). More likely, these events confer a permissive effect, whereby hypertrophic responses are facilitated by the favorable anabolic environment.

Responses and Adaptations of Myokines

The term *myokine* is commonly used to describe cytokines that are expressed and locally secreted by skeletal muscle to interact in an autocrine/paracrine fashion as well as reaching the circulation to exert influence on other tissues (171, 172). Exercise training results in the synthesis of these substances within skeletal muscle, and an emerging body of evidence indicates that they can have unique effects on skeletal muscle to promote anabolic or catabolic processes (see table 1.3) (153, 177, 206). The actions of myokines are purported to be biphasic, where they first bind to cellular receptors and then regulate signal transduction via an array of intracellular messengers and transcription factors (162). Myokine production provides a conceptual basis for clarifying how muscles communicate intracellularly and with other organs. There are dozens of known myokines, and new variants continue to be identified. This section addresses some of the better studied of these agents and their effects on muscle hypertrophy.

Mechano Growth Factor

Mechano growth factor (MGF) is widely considered necessary for compensatory muscle growth, even more so than the systemic IGF-1 isoforms (88). As previously mentioned, resistance training acutely upregulates MGF mRNA expression (107). Current theory suggests that this event helps to kick-start post-exercise muscle recovery by facilitating the local repair and regeneration following myotrauma (71). In support of this view, Bamman and colleagues (20) recruited 66

TABLE 1.3 Primary Myokines and Their Respective Actions

Myokine	Actions
Mechano growth factor (MGF)	Believed to kick-start the growth process following resistance training. Upregulates anabolic processes and downregulates catabolic processes. Involved in early-stage satellite cell responses to mechanical stimuli.
Interleukins (ILs)	Numerous ILs are released to control and coordinate the post-exercise immune response. IL-6, the most studied of the ILs, appears to carry out hypertrophic actions by inducing satellite cell proliferation and influencing satellite cell–mediated myonuclear accretion. Emerging research indicates that IL-15 may be important to exercise-induced anabolism, although evidence remains somewhat preliminary. Other ILs also have been postulated to play a role in hypertrophy, including IL-4, IL-7, IL-8, and IL-10, although evidence on their exercise-induced effects remains equivocal.
Myostatin	Serves as a negative regulator of muscle growth. Acts to reduce myofibrillar protein synthesis and may also suppress satellite cell activation.
Hepatocyte growth factor (HGF)	Activated by nitric oxide synthase and possibly calcium–calmodulin as well. HGF is believed to be critical to the activation of quiescent satellite cells.
Leukemia inhibitory factor (LIF)	Upregulated by the calcium flux associated with resistance exercise. Believed to act in a paracrine fashion on adjacent satellite cells to induce their proliferation.

men and women of various ages to undertake 16 weeks of lower-body resistance training. Based on their hypertrophic response to the program, subjects were then categorized as either extreme responders (mean myofiber hypertrophy of 58%), moderate responders (mean myofiber hypertrophy of 28%), or nonresponders (no significant increase in myofiber hypertrophy). Muscle biopsy analysis showed a differential MGF expression across clusters: Whereas MGF levels increased by 126% in those classified as extreme responders, concentrations remained virtually unchanged in nonresponders. These results imply that transient exercise-induced increases in MGF gene expression serve as critical cues for muscle remodeling and may be essential to producing maximal hypertrophic gains.

MGF is purported to regulate muscle growth by several means. For one, it appears to directly stimulate muscle protein synthesis by the phosphorylation of *p70S6 kinase* (a serine/threonine kinase that targets the S6 ribosomal protein; phosphorylation of S6 causes protein synthesis at the ribosome; it is also written as p70S6K or $p70^{S6K}$) via the PI3K/Akt pathway (see chapter 2) (7, 8, 156). MGF also may elevate muscle protein synthesis by downregulating the catabolic processes involved in proteolysis. Evidence indicates that the activation of MGF suppresses FOXO nuclear localization and transcriptional activities, thereby helping to inhibit protein breakdown (73). These combined anabolic and anticatabolic actions are thought to heighten the post-exercise hypertrophic response.

MGF also is believed to influence hypertrophic adaptations by mediating the satellite cell response to exercise training. Although systemic IGF-1 promotes later-stage effects on satellite cell function, local expression of the peptide has been shown to be involved primarily in the initial phases. This is consistent with research demonstrating that MGF regulates extracellular signal–regulated kinases (ERK1 and ERK2; also abbreviated as ERK1/2), whereas the systemic isoforms do not. It is also consistent with research demonstrating that MGF is expressed earlier than hepatic (liver)-type IGF-1 following exercise (21, 72). Accordingly, MGF appears to be involved in inducing satellite cell activation and proliferation (92, 250), but not differentiation (250). This observation suggests that MGF increases the number of myoblasts available for post-exercise repair as well as facilitating the replenishment of the satellite cell pool. However, other research challenges MGF's role in satellite cell function. Fornaro and colleagues (66) demonstrated that high concentrations of MGF failed to enhance proliferation or differentiation in both mouse C2C12 murine myoblasts and human skeletal muscle myoblasts, as well as primary mouse muscle stem cells. Interestingly, mature IGF-1 promoted a strong proliferative response in all cell types. The discrepancies between this study and previous work are not readily apparent.

Interleukins

The *interleukins* (ILs) are a class of cytokines released by numerous bodily tissues to control and coordinate immune responses. The most studied of these isoforms is IL-6, an early-stage myokine believed to play an important and perhaps even critical role in exercise-induced muscular growth. This contention is supported by research showing that IL-6 deficient mice display an impaired hypertrophic response (206). IL-6 is also considered an important growth factor for human connective tissue, stimulating collagen synthesis in healthy tendons (15). Such actions enhance the ability of muscle tissue to endure high levels of mechanical stress.

Resistance training acutely upregulates IL-6 by up to 100-fold, and exercise-induced metabolic stress may further stimulate its production (62). Moreover, the magnitude of post-exercise IL-6 expression significantly correlates with hypertrophic adaptations (140). Contracting skeletal muscles account for a majority of circulating IL-6; additional sources are synthesized by connective tissue, adipocytes, and the brain (163). The appearance of IL-6 in the systemic circulation precedes that of other cytokines, and the magnitude of its release is by far more prominent. It was initially thought that muscle damage was a primary mediator of the IL-6 response. This seems logical, given that damage to muscle tissue initiates an inflammatory cas-

cade. However, emerging evidence indicates that myodamage is not necessary for its exercise-induced release. Instead, damaging exercise may result in a delayed peak and a slower decrease of plasma IL-6 during recovery (163).

The primary hypertrophic actions of IL-6 appear to be related to its effects on satellite cells, both by inducing proliferation (102, 229) and by influencing satellite cell–mediated myonuclear accretion (206). There also is evidence that IL-6 may directly mediate protein synthesis via activation of the Janus kinase/signal transducer and activator of transcription (JAK/STAT), ERK1/2, and PI3K/Akt signal transduction pathways (see chapter 2) (184).

IL-15 is another myokine that has received considerable interest as having a potential role in skeletal muscle growth. Muscle is the primary source of IL-15 expression, and exercise regulates its production. Resistance training, in particular, has been shown to acutely elevate IL-15 protein levels, apparently through its release via microtears in muscle fibers as a result of inflammation, oxidative stress, or both (177, 186). Type II fibers show a greater increase in IL-15 mRNA levels than Type I fibers (152).

Early animal research suggested that IL-15 exerted anabolic effects by acting directly on differentiated myotubes to increase muscle protein synthesis and reduce protein degradation (177). A polymorphism in the gene for IL-15 receptor was found to explain a relatively large proportion of the variation in muscle hypertrophy (186). Moreover, recombinant IL-15 administration in healthy growing rats produced more than a 3-fold decrease in the rate of protein breakdown, leading to an increase in muscle weight and contractile protein accretion (177). However, recent research is conflicting as to whether IL-15 causes the hypertrophic adaptations originally thought. For one, IL-15 mRNA correlates poorly with protein expression. In addition, hypertrophic effects of IL-15 have been observed solely in diseased rodents. Quinn and colleagues (176) demonstrated that transgenic mice constructed to oversecrete IL-15 substantially reduced body fat but only minimally increased lean tissue mass. Muscular gains were limited to the slow/oxidative soleus muscle, whereas the fast/glycolytic extensor digitorum longus muscle had slight decreases in hypertrophy. Given this emerging evidence, it has been hypothesized that IL-15 serves to regulate the oxidative and fatigue properties of skeletal muscle as opposed to promoting the accretion of contractile proteins (172). In contrast, Pérez-López and colleagues (165) demonstrated an upregulation of skeletal muscle gene expression after a resistance training bout, with an association between its expression and early-stage elevations in post-exercise myofibrillar protein synthesis. Despite the burgeoning research on this myokine, the extent of its hypertrophic role during regimented resistance training remains unclear.

Research on other ILs are limited at this time. IL-10 has been implicated as an important mediator of processes that drive myoblast proliferation and myofiber growth (171). Other evidence suggests that IL-4 is involved in myogenic differentiation (194). IL-7 also is believed to play a role in muscle hypertrophy and myogenesis (164), and IL-8 has been shown to have potent anticatabolic effects on skeletal muscle (138). Substantially more research is needed for developing a complete understanding of the roles of each of these IL isoforms (and perhaps others) with respect to exercise-induced muscular adaptations.

The acute effects of resistance exercise on ILs must be differentiated from chronically elevated levels of these cytokines. Evidence indicates that chronic low-grade inflammation, as determined by increased circulating concentrations of pro-inflammatory cytokines, is correlated with the age-related loss of muscle mass (137). Moreover, hospitalized patients exhibiting chronically high levels of inflammation display a reduced capacity to increase muscle mass following performance of a regimented resistance training program (155). This is consistent with evidence that while acute exercise-induced increases in IL-6 induce myogenic progression, persistent elevations of this myokine suppress muscle protein synthesis (151). Reducing chronically elevated inflammatory levels with nonsteroidal anti-inflammatory drugs has been shown to restore muscle protein

anabolism and significantly reduce muscle loss in aging rats (187). Moreover, physical activity displays an inverse correlation with low-grade systemic inflammation (163): The acute elevation of ILs enhances anabolism, whereas the suppression of chronic IL production mitigates catabolic processes.

Myostatin

Myostatin (MSTN), a member of the transforming growth factor-β superfamily, is recognized as a powerful negative regulator of developing muscle mass (108). The MSTN gene is expressed almost exclusively in muscle fibers throughout embryonic development as well as in adult animals (200). A mutation of the MSTN gene has been shown to produce marked hypertrophy in animals. A breed of cattle known to be null for the MSTN gene, called the Belgian Blue, displays a hypermuscular appearance (figure 1.12), so much so that they are popularly referred to as Schwarzenegger cattle after the champion bodybuilder. Targeted disruption of the MSTN gene in mice causes a doubling of skeletal muscle mass (136), ostensibly from a combination of hyperplasia and hypertrophy. Moreover, MSTN inhibition increases myofiber hypertrophy by 20% to 30% in both young and old mice in the absence of structured exercise (129, 175).

The regulatory effects of MSTN are present in humans, as exemplified in a case report of an infant who appeared extraordinarily muscular at birth, with protruding thigh muscles (200). The child's development was followed over time, and at 4.5 years of age he continued to display superior levels of muscle bulk and strength. Subsequent genetic analysis revealed that the child was null for the MSTN gene, which conceivably explains his hypermuscularity.

There is conflicting evidence as to the quality of muscle tissue in MSTN deficiencies. Racing dogs found to be null for the MSTN gene were significantly faster than those carrying the wild-type genotype, suggesting a clear performance advantage (143). Alternatively, other research shows that a mutation of the MSTN gene in mice is associated with impaired calcium release from the sarcoplasmic reticulum (31). So although these mice are hypermuscular in appearance, the increased muscle mass does not translate into an increased ability to produce force. There also is evidence that MSTN dysfunction negatively affects hypertrophy in muscles comprised of primarily slow-twitch fibers, which in turn may have a detrimental

FIGURE 1.12 Belgian Blue, a breed of cattle known to be null for the myostatin gene.

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impact on muscular endurance (139). At this point, the functional implications of alterations in MSTN remain undetermined.

MSTN carries out its actions via downstream signaling of the transcription factors SMAD2 and SMAD3, which in turn negatively regulate hypertrophy independent of the catabolic enzyme muscle ring finger protein-1 (MuRF-1). Early research indicated that atrophic actions of MSTN were attributed to an inhibition of satellite cell activation, thus impairing protein synthetic capacity (135). Moreover, in vitro research showed that MSTN blunted satellite cell proliferation and differentiation (255). However, subsequent research has refuted these findings, showing instead that MSTN inhibition increases muscle mass primarily by acting on muscle fibers as opposed to satellite cells, thereby increasing the cytoplasmic volume to DNA ratio (243). The body of evidence appears to suggest that the primary mechanism of MSTN action in the postnatal period is the modulation of myofibrillar muscle protein synthesis (6), although it may still play a minor role in regulating satellite cell function (86). The negative regulation of muscle protein synthesis is thought to occur via a combined inhibition of the Akt/mTOR pathway (see chapter 2) as well as downregulation of both calcineurin signaling and the transcription factors MyoD and myogenin (236). Myostatin-induced inhibition of mTOR is self-perpetuating because this downregulation in turn further amplifies MSTN signaling (70).

In addition to acutely upregulating numerous growth-related factors, resistance training also downregulates inhibitory factors, including MSTN (107). Untrained people show modest decreases in MSTN following a resistance exercise bout, and these reductions are more than 3-fold greater, with consistent resistance training experience (148). Moreover, an inverse relationship was shown between thigh muscle mass and the resistance training–induced load-mediated decrease in myostatin mRNA expression, indicating that those larger muscles were more responsive to reductions in MSTN (107). Other research also shows a correlation between the downregulation of MSTN and increases in muscle cross-sectional area following resistance exercise (179), although these findings are not universal (63). Thus, the specific role of MSTN with respect to its hypertrophic effects during resistance training remains to be fully elucidated.

Other Myokines

A number of additional myokines have been identified, and emerging evidence indicates that many of these substances may play a role in hypertrophic adaptations. Perhaps the most intriguing of these is *hepatocyte growth factor* (HGF), which exerts mitogenic actions on numerous bodily tissues, including muscle. Evidence shows that HGF is critical for the activation of dormant satellite cells (5). To date, HGF is the only myokine shown to stimulate quiescent satellite cells to enter the cell cycle early both in vitro and in vivo (223).

The active form of HGF is present in the extracellular compartment of uninjured skeletal muscle (221), and it is activated by mechanical signaling via the dystrophin-associated protein complex (5). Muscular contractions alter this complex, leading to nitric oxide synthase activation, which stimulates the release of HGF from the extracellular matrix and facilitates its interaction with receptors on satellite cells (5). There is also evidence that calcium–calmodulin signaling mediates HGF release from the matrix independent of nitric oxide production (222). Evidence shows that HGF is critical for the activation of inactive satellite cells (5). Interestingly, chronically high levels of HGF are associated with the upregulation of MSTN mRNA, which in turn may have a negative effect on the proliferative response and return satellite cells to quiescence (6). These data highlight the fine regulatory role that HGF seems to have in the growth process.

Leukemia inhibitory factor (LIF) is another myokine that has been shown to play a role in muscle hypertrophy (215). During exercise, skeletal muscle markedly upregulates the expression of LIF mRNA, likely as a result of fluctuations in intracellular calcium concen-

trations (34). Mice null for the LIF gene were incapable of increasing muscle size following muscular overload, but the growth response was restored following recombinant LIF administration (215). It is hypothesized that LIF exerts hypertrophic effects primarily by acting in a paracrine fashion on adjacent satellite cells, inducing their proliferation while preventing premature differentiation (34).

Many additional myokines with potential hypertrophic effects have been identified in the literature, including fibroblast growth factor, brain-derived neutrophic factor, tumor necrosis factor, follistatin, platelet-derived growth factor-BB, vascular endothelial growth factor, and chitinase-3-like protein 1, among others.

KEY POINT

Myokines are autocrine or paracrine agents that exert their effects directly on muscle tissue as a result of mechanical stimulation. Numerous myokines have been identified, although the specific roles of the substances and their interactions with one another have yet to be elucidated.

Myokines are a relatively new area of research, and the study of these substances is continually evolving. Over the coming years, we should have a much greater understanding of their scope and effects on muscle growth.

TAKE-HOME POINTS

- Early-phase adaptations to resistance training are primarily related to neural improvements including greater recruitment, rate coding, synchronization, and doublet firing. The extent and temporal course of neural adaptations depend on the degrees of freedom and complexity of the movement patterns.
- Muscular adaptations are predicated on net protein balance over time. The process is mediated by intracellular anabolic and catabolic signaling cascades.
- Hypertrophy can occur in series or in parallel, or both. The primary means by which muscles increase in size following resistance training is through parallel hypertrophy. Resistance training does promote changes in sarcoplasmic fractions, but it is not clear whether these adaptations are practically meaningful from a hypertrophic standpoint, nor is it known whether different training protocols elicit differential effects on the extent of these changes. There is contradictory evidence as to whether hyperplasia occurs as a result of traditional resistance training; if any fiber splitting does occur, the overall impact on muscle size appears to be relatively minimal.
- Satellite cells appear to be crucial to maximizing the hypertrophic response to resistance training. The primary role of satellite cells appears to be their ability to retain a muscle's mitotic capacity by donating nuclei to existing myofibers. Satellite cells also are involved in the repair and remodeling of muscle tissue, including the co-expression of myogenic regulatory factors that mediate growth-related processes. Additional hypertrophic effects of satellite cells may lie in their regulatory role in the remodeling of extracellular matrix components.

- The endocrine system is intricately involved in the regulation of muscle mass. The chronic production of testosterone, growth hormone, IGF-1, and other anabolic hormones influences protein balance to bring about changes in resistance training–mediated muscular adaptations. Although the manipulation of resistance training variables can acutely elevate systemic levels in the immediate post-workout period, it is not clear whether these transient hormonal spikes play a role in the hypertrophic response; if there are any such effects, they appear to be of relatively minor consequence and most likely permissive in nature.
- Myokines are important players in exercise-induced muscular adaptations. These autocrine/paracrine agents exert their effects directly on muscle tissue as a result of mechanical stimulation. Numerous myokines have been identified, although the specific roles of the substances and their interactions with one another have yet to be elucidated.

chapter 2

Mechanisms of Hypertrophy

Increased muscle protein accretion following resistance exercise has been attributed to three primary mechanisms: mechanical tension, metabolic stress, and muscle damage (240). This chapter addresses each of these mechanisms and the theoretical rationale for their promotion of a hypertrophic response.

Mechanical Tension

Skeletal muscle is highly responsive to alterations in mechanical loading. Accordingly, a number of researchers have surmised that *mechanical tension* is the primary driving force in the hypertrophic response to regimented resistance training (77, 88) and at the very least initiates critical hypertrophy-related intracellular signaling following resistance exercise (226). In simple terms, mechanical tension can be defined as a force normalized to the area over which it acts, with units expressed in either newtons per square meter or pascals (31). Mechanical tension alone has been shown to directly stimulate mTOR (113), possibly through activation of the extracellular signal–regulated kinase/tuberous sclerosis complex 2 (ERK/TSC2) pathway (188). It is theorized that these actions are mediated via the synthesis of the lipid second messenger phosphatidic acid by phospholipase D (113, 206). There also is evidence that phosphatidic acid can phosphorylate $p70^{S6K}$ independent of mTOR (151), presenting another potential avenue whereby mechanical stimuli may directly influence muscle protein synthesis.

Research indicates that mechanosensors are sensitive to both the magnitude and temporal aspects of loading. Using an *in situ* model (i.e., examining an intact muscle within the animal), Martineau and Gardiner (167) subjected rat plantaris muscles to peak concentric, eccentric, isometric, and passive tensions. Results showed tension-dependent phosphorylation of c-Jun N-terminal kinase (JNK) and ERK1/2; eccentric actions generated the greatest effect, and passive stretch generated the least. Peak tension was determined to be a better predictor of mitogen-activated protein kinase (MAPK) phosphorylation than either time under tension or rate of tension development. In a follow-up study by the same lab (168), an in situ evaluation of the rat gastrocnemius muscle showed a linear relationship between time under tension and the signaling of JNK, whereas the rate of change of tension showed no effect. This suggests that time under tension is an important parameter for muscle hypertrophic adaptations. In support of these findings, Nader and Esser (193) reported increased activation of $p70^{S6K}$ following both high-intensity and low-intensity electrical stimuli of the rat hind limb; however, the response was not as prolonged following the low-intensity protocol. Similarly, in vitro research shows a magnitude-dependent effect on $p70^{S6K}$ signaling when mouse C2C12 myoblasts are subjected to biaxial strain (74).

Mechanosensors also appear to be sensitive to the type of load imposed on muscle tissue. Stretch-induced mechanical loading elicits the deposition of sarcomeres longitudinally (i.e., in series), whereas dynamic muscular actions increase cross-sectional area in parallel with the axes (74). Moreover, the hypertrophic response

can vary based on the type of muscle action. Isometric and eccentric actions stimulate the expression of distinct genes in a manner that cannot be explained by differences in the magnitude of applied mechanical force (74). These examples highlight the intricate complexity of mechanosensors and their capacity to distinguish between types of mechanical information to produce an adaptive response. What follows is a discussion of how mechanical forces regulate muscle hypertrophy via mechanotransduction and associated intracellular signaling pathways.

KEY POINT

Mechanical tension is the most important factor in training-induced muscle hypertrophy. Mechanosensors are sensitive to both the magnitude and the duration of loading, and these stimuli can directly mediate intracellular signaling to bring about hypertrophic adaptations.

Mechanotransduction

Exercise has a profound effect on muscle protein balance. When muscles are mechanically overloaded and then provided with appropriate nutrients and recovery, the body initiates an adaptive response that results in the accretion of muscle proteins. Transmission of mechanical forces from the sarcomeres to tendons and bones occurs both longitudinally along the length of the fiber and laterally through the matrix of fascia tissue (259). The associated response is accomplished through a phenomenon called *mechanotransduction*, whereby mechanical forces in muscle are converted into molecular events that mediate intracellular anabolic and catabolic pathways (see figure 2.1) (308).

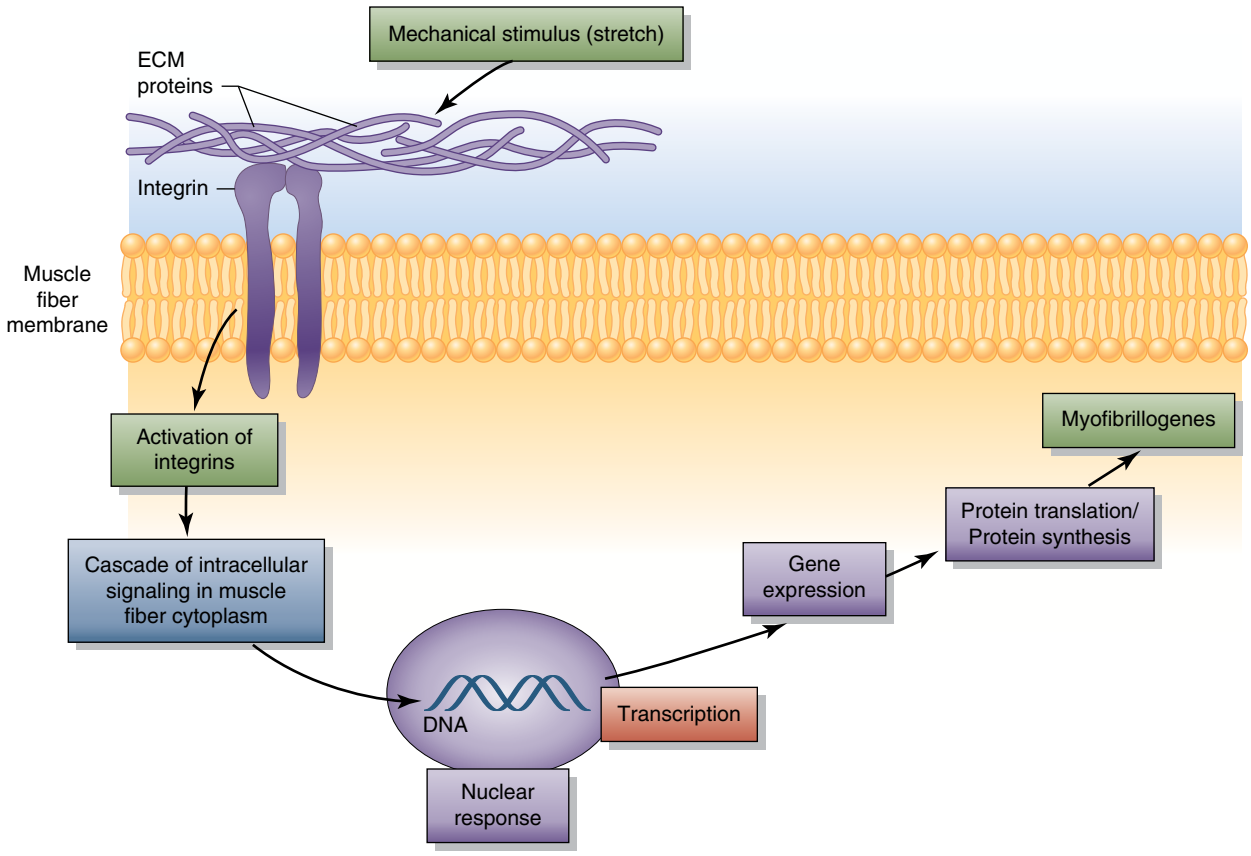

FIGURE 2.1 The process of mechanotransduction.

Adapted from P.G. De Deyne, "Application of Passive Stretch and Its Implications for Muscle Fibers," *Physical Therapy* 81, no. 2 (2001): 819-827.

A diverse array of tissue and substances help to carry out mechanotransduction, including stretch-activated ion channels, caveolae, integrins, cadherins, growth factor receptors, myosin motors, cytoskeletal proteins, nuclei, and the extracellular matrix (74). These mechanosensory elements do not function independently, but rather act in a coordinated manner with structural components such as the cytoskeleton to elicit intracellular events (74). Central to the process are mechanosensors that detect mechanical tension and transduce the stimuli into chemical signals within the myofiber. Integrins have been identified as a primary mechanosensor. These receptors reside at the cell surface and interact with the extracellular matrix to facilitate the transmission of mechanical and chemical information from the outside to the inside of the cell (307, 308). Integrins mediate intracellular signal transduction as part of focal adhesion complexes (i.e., costameres), which are sarcolemmal proteins that bridge the connection between the extracellular matrix and the cytoskeleton. Focal adhesion complexes can directly enhance protein translation via activation of ribosomal proteins, and their disruption impairs intracellular anabolic signaling (173). Emerging evidence shows that an enzyme called focal adhesion kinase (FAK) serves as a key player in signal initiation (48). The expression of FAK displays load-dependent characteristics whereby its activation is suppressed during unloading and heightened during mechanical overload, highlighting the mechanosensitive role of FAK in exercise-induced hypertrophy (9).

Other stimuli and sensors have been surmised to play a role in hypertrophic adaptations. For example, emerging evidence implicates titin as a primary mechanosensor, and the level of signaling depends on its passive stiffness: High stiffness mediates a stronger anabolic response whereas low stiffness moderates the response (285). Moreover, G protein–coupled receptors, which show structural similarity to integrin receptors, are proposed as a potential link between mechanical force transduction and upregulation of intracellular anabolic pathways (301). It also has been hypothesized that the "flattening" of myonuclei during mechanical loading may act as a sensory signal for various growth-related proteins (e.g., YAP) to translocate from the cytosol to the nucleus and thus initiate anabolism (294), although this theory remains speculative. Overall, our understanding of the stimuli and sensors involved in mechanotransduction is poorly characterized; the topic will be an important area of future research.

Once forces are transduced, intracellular enzymatic cascades carry out signaling to downstream targets that ultimately shift muscle protein balance to favor synthesis over degradation. Certain pathways act in a permissive role, whereas others directly mediate cellular processes that influence mRNA translation and myofiber growth (172). A number of primary anabolic signaling pathways have been identified, including the PI3K/Akt pathway, MAPK pathways, calcium-dependent pathways, and the phosphatidic acid pathway (see figure 2.2), among others. Although these pathways may overlap at key regulatory steps, there is evidence they may be interactive rather than redundant (276).

Alternatively, muscle catabolism is regulated by four proteolytic systems: autophagy-lysosomal, calcium-dependent calpains, the cysteine protease caspase enzymes, and the ubiquitin–proteasome system (211). The 5′-AMP-activated protein kinase (AMPK) pathway is believed to act as a metabolic master switch in these systems. It is activated in response to environmental stressors (e.g., exercise) to restore cellular energy balance via an increase of catabolic processes and a suppression of anabolic processes (see figure 2.3 on page 34). The MSTN-SMAD pathway also is considered a strong catabolic regulator of muscle protein accretion.

Signaling Pathways

This section provides a general overview of the primary anabolic intracellular signaling pathways and their significance to skeletal muscle hypertrophy. Although huge strides have been made to elucidate these pathways, our understanding of their relative importance is limited at this time.

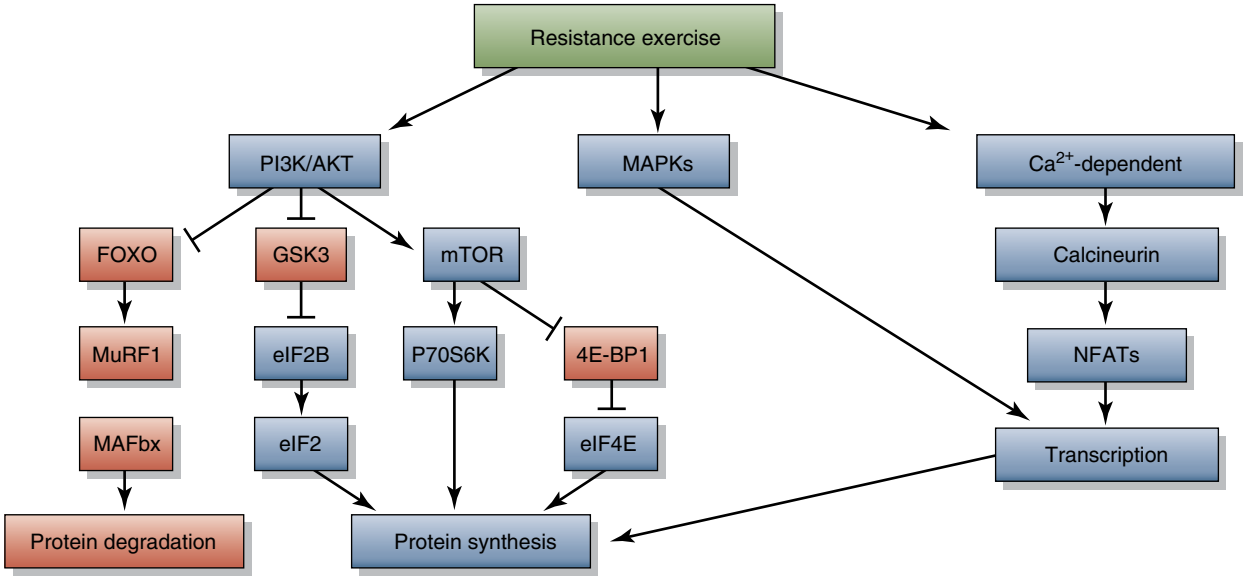

FIGURE 2.2 Primary anabolic intracellular signaling pathways.

Reprinted from B.J. Schoenfeld, "Potential Mechanisms for a Role of Metabolic Stress in Hypertrophic Adaptations to Resistance Training," *Sports Medicine* 43, no. 3 (2013): 179-194, by permission of Springer Nature.

KEY POINT

Numerous intracellular signaling pathways have been identified in skeletal muscle including PI3K/Akt, MAPK, phosphatidic acid, AMPK, and calcium-dependent pathways. The serine/threonine kinase mTOR has been shown to be critical to resistance training–induced hypertrophic adaptation.

PI3K/Akt Pathway

The phosphatidylinositol 3-kinase (PI3K)/Akt pathway is considered a master network for regulating skeletal muscle growth (20, 125, 274). Akt, also known as protein kinase B (PKB), acts as a molecular upstream nodal point that functions both as an effector of anabolic signaling and a dominant inhibitor of catabolic signals (279). Multiple isoforms of Akt have been identified in skeletal muscle (Akt1, Akt2, Akt3), and each has a distinct physiological role. Of these isoforms, Akt1 appears to be most responsive to mechanical stimuli (307). Early research indicated that high mechanical intensities were required to activate Akt; however, subsequent studies demonstrate evidence to the contrary (307).

A primary means by which Akt carries out its actions is by signaling mTOR, which has been shown to be critical to hypertrophic adaptations induced by mechanical loading. mTOR, named because the pharmacological agent rapamycin antagonizes its growth-promoting effects, exists in two functionally distinct signaling complexes: mTORC1 and mTORC2. Only mTORC1 is inhibited by rapamycin (203), and this complex was originally thought to be responsible for mTOR's hypertrophic regulatory actions; however, recent research indicates that rapamycin-insensitive mTORC2 also plays a role in load-induced anabolism (202). Some evidence shows that early increases in muscle protein synthesis are regulated by mTORC1, while continued elevations at later time points involve rapamycin-insensitive or perhaps even mTOR-independent mechanisms (92). It should be noted that mTOR is regulated by a variety of inputs and functions as an energy and nutrient sensor: Elevated energy levels promote its activation while reduction in energy levels and nutrient availability result in its suppression (19).

Once activated, mTOR exerts its effects by turning on various downstream anabolic

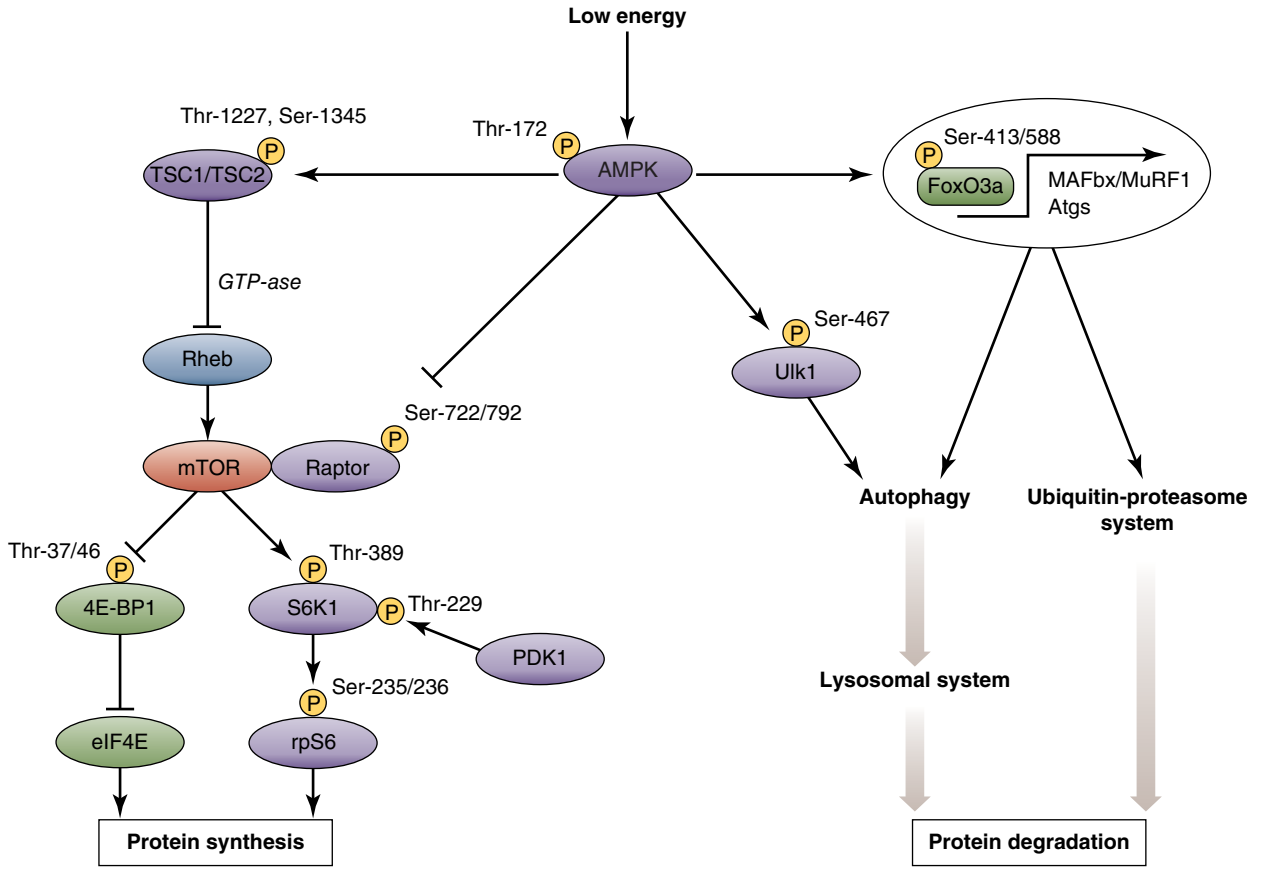

FIGURE 2.3 Primary proteolytic pathways.

Reprinted by permission from A.M.J. Sanches et al., "The Role of AMP-Activated Protein Kinase in the Coordination of Skeletal MuscleTurnover and Energy Homeostasis," *American Journal of Physiology—Cell Physiology* 303, no. 5 (2012): C475-C485.

effectors. A primary target of mTOR is $p70^{S6K}$, which plays an important role in the initiation of mRNA translation (90). mTOR also exerts anabolic effects by inhibiting eukaryotic initiation factor 4E-binding protein 1 (eIF4EB1), a negative regulator of the eIF4E protein that is a potent mediator of protein translation (86). Interestingly, persistently elevated basal levels of mTOR have been shown to impair fast-twitch fiber growth in mice and contribute to anabolic resistance in elderly humans; thus, it has been postulated that mTOR mediates hypertrophy within a given range, and deviations outside of this range may be detrimental to the growth process (56, 92).

Signaling through PI3K/Akt also regulates mTOR-independent growth regulatory molecules to directly inhibit catabolic processes. For one, Akt phosphorylates FOXO proteins—a subgroup of the Forkhead family of transcription factors that encourage atrophy—which induces their translocation from the nucleus to the cytoplasm (90, 106). The cytoplasmic sequestration of FOXO proteins, in turn, blocks upregulation of the ubiquitin ligases MuRF-1 and atrogin-1 (also called MAFbx) and thus helps to lessen the extent of muscle protein breakdown. Indeed, activation of Akt was found to be sufficient to impair increases in the atrophy-associated enzymes MuRF-1 and atrogin-1 transcription via FOXO phosphorylation (86). Akt also suppresses the activation of glycogen synthase kinase 3 beta (GSK3β), which blocks protein translation initiated by the eIF2B protein (86, 206). As opposed to mTORC1, which regulates the translation of a small subset of mRNAs, eIF2B is believed to control the translation initiation of virtually all

mRNAs, and therefore acts to regulate global rates of protein synthesis (90). Thus, the anticatabolic actions of PI3K/Akt may indirectly provide an even more potent stimulus for growth than its anabolic effects.

The hypertrophic properties of PI3K/Akt are incontrovertible. Induction of the pathway has been shown to mediate protein translation both in vitro and in vivo, as well as promote myoblast differentiation (86). However, recent research indicates that PI3K/Akt activation is not obligatory for increases in muscle hypertrophy (292). Resistance exercise activates $p70^{S6K}$ in humans via an Akt-independent pathway (60, 170, 271). Moreover, mTOR can be activated via a variety of intracellular signals other than PI3K/Akt, indicating that the pathways influencing growth are complex and diverse. The primary anabolic property of Akt1 during load-induced hypertrophy may be in its ability to regulate satellite cell proliferation (189).

MAPK Pathways

Mitogen-activated protein kinase (MAPK) is a primary regulator of gene expression, redox status, and metabolism (141). With respect to exercise-induced muscle growth, MAPK is believed to link cellular stress with an adaptive response in myofibers, modulating their growth and differentiation (231). It is theorized that the maximal anabolic response to resistance exercise is at least in part reliant on coactivation of the MAPK and mTORC1 signaling cascades (210). Moreover, MAPK has been implicated in the regulation of ribosome biogenesis, which is critical to sustained increases in muscle growth (67). Three distinct MAPK signaling modules are associated with mechanically stimulated hypertrophic adaptations: ERK1/2, p38 MAPK, and JNK. Activation of these modules depends on the type, duration, and intensity of the stimulus.

ERK1/2 is upregulated by both aerobic endurance and resistance training, and the magnitude of its phosphorylation correlates with the intensity of exercise (141). Studies investigating the role of ERK1/2 in the regulation of muscle mass have been somewhat conflicting. On one hand, there is evidence that it mediates satellite proliferation and induces muscle protein synthesis (85); on the other hand, some research shows opposite effects (61). That said, early signaling of mTORC1 likely occurs through activation of the ERK/TSC2 pathway (188). Whereas Akt and ERK1/2 both stimulate mTOR to a similar extent, their combined effects lead to an even greater stimulation compared to either alone (305). Moreover, the two pathways appear to be synergistic to satellite cell function; ERK1/2 stimulates cell proliferation, and PI3K facilitates differentiation (101).

Activation of p38 MAPK occurs primarily following aerobic endurance exercise. Four p38 isoforms have been identified (p38α, p38β, p38δ, and p38γ). Of these isoforms, p38γ is specific to muscle tissue, whereas p38α and p38β are expressed throughout the body; p38δ does not appear to be involved with muscular actions; p38γ is preferentially upregulated in slow-twitch fibers while remaining largely inactive in fast-twitch fibers (73). Moreover, a loss of p38γ in rat and mouse models is associated with a decrease in slow-twitch fiber size and no change in fast-twitch fibers (73). As opposed to directly binding to DNA, p38 MAPK mediates transcription of target genes by activating other transcription factors (96). There is evidence that p38 may regulate hypertrophy by stimulating Notch signaling, which has been deemed essential for the activation, proliferation, and progression of myogenic satellite cells necessary for muscle regeneration and repair (25).

Of all the MAPK modules, JNK appears to be the most sensitive to mechanical tension, and it is particularly responsive to eccentric actions. Contraction-induced phosphorylation of JNK correlates with a rapid rise in mRNA of transcription factors that mediate cell proliferation and DNA repair (7, 8), indicating a role in muscle regeneration following intense exercise. Moreover, JNK phosphorylation displays a linear increase, with heightened levels of contractile force (141). However, the specific role of JNK in exercise-induced muscle hypertrophy remains undetermined. Some researchers have deemed JNK a molecular switch that, when activated, stimulates a hypertrophic response and, when suppressed, induces smaller, more oxidative muscle fibers; these effects were found to be regulated, at least in part, by myostatin

inhibition (152). However, other research suggests that inhibition of JNK actually enhances muscle protein accretion (25), so its precise role in muscle anabolism remains somewhat unclear.

The interplay between the MAPK modules and their potential hypertrophic synergism with one another has yet to be established. In response to synergist ablation of the rat gastrocnemius, p38α MAPK phosphorylation occurred early following overload and remained elevated in both slow-twitch soleus and fast-twitch plantaris muscles over the ensuing 24-hour study period. Conversely, ERK2 and JNK phosphorylation increased transiently post-ablation; levels returned to that of sham-operated controls (placebo-controlled surgical interventions) by 24 hours. The implications of these findings are not clear at present.

Calcium-Dependent Pathways

Intracellular calcium plays an important role in signal transduction in a variety of cell types, including skeletal muscle (38). An increase in myoelectrical activity substantially elevates calcium levels within myofibers, and this alteration is considered to be a primary mediator of skeletal muscle gene expression (38). Increased intracellular calcium levels have been shown to amplify protein synthesis via TORC1 signaling, although the mechanism of action is as yet unknown (173). Moreover, elevations in extracellular ATP promote muscle hypertrophy via an increase in intracellular calcium levels, leading to subsequent downstream anabolic signaling in rodents (124). Intriguingly, the hypertrophy occurred in the soleus but not the plantaris muscles, suggesting that calcium-dependent effects are specific to slow-twitch fibers.

Various calcium-dependent pathways have been implicated in the control of skeletal muscle mass. Calcineurin, a calcium-regulated phosphatase, is believed to have a particularly important role in muscular adaptations. Calcineurin is activated by a sustained increase in intracellular calcium levels. Once aroused, it acts on various downstream anabolic effectors, including myocyte-enhancing factor 2 (MEF2), GATA transcription factors, and nuclear factor of activated T cells (NFAT) (183). Calcineurin has been shown to promote hypertrophy in all fiber types, whereas its inhibition prevents growth even when muscles were subjected to overload (57, 58). Early evidence suggested that, along with PI3K/Akt signaling, activation of calcineurin was required for IGF-1–mediated hypertrophic adaptations (119). It was hypothesized that these effects were expressed via activation of NFAT, which in turn mediated the signaling of transcriptional regulators such as proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) and striated muscle activator of Rho signaling (STARS) (162, 169). However, subsequent research challenged these findings, indicating that calcineurin in muscle was primarily responsible for producing a shift toward a slower phenotype (195, 267). When considering the body of literature as a whole, evidence suggests both correlative and causal links between calcineurin and muscle fiber size, especially in slow-twitch fibers (119). That said, muscle growth does not appear to be dependent on calcineurin activity (14), and the role (if any) that the enzyme plays in the hypertrophic response to exercise overload is unclear.

The calcium-calmodulin-dependent kinases (i.e., CaMKII and CaMKIV) also have a prominent role in muscle plasticity. CaMKII and CaMKIV have multiple isoforms that detect and respond to calcium signals via multiple downstream targets (38). CaMKII is activated by both acute and long-duration exercise, indicating that it mediates muscle growth as well as mitochondrial biogenesis (38). Interestingly, increases in one of the CaMKII isoforms (CaMKIIγ) occurs during muscle atrophy, leading to the possibility that it is upregulated as a compensatory response to counter the wasting process (38).

Phosphatidic Acid Pathway

Phosphatidic acid (PA) is a lipid second messenger that regulates a diverse array of cellular processes, including muscle growth in response to mechanical load. The activation of PA is mediated via several classes of enzymes. In particular, it is synthesized by phospholipase D1 (PLD1), which hydrolyzes phosphatidylcholine into PA and choline. Once activated, PA exerts

effects on both protein synthesis and proteolysis. This is principally accomplished by its direct binding to mTOR and then activating $p70^{S6K}$ activity (248, 307). PA also can phosphorylate $p70^{S6K}$ in an mTOR-independent manner, presenting yet another path whereby mechanical stimuli may directly drive anabolic processes (151). In addition, overexpression of PLD1 is associated with a decrease in catabolic factors such as FOXO3, atrogin-1, and MuRF-1 (91). Suppression of these atrophy-related genes is believed to be due to Akt phosphorylation and subsequent activation of mTORC2. Thus, PLD1 carries out anabolic and anticatabolic actions through varied intracellular mechanisms.

PA is highly sensitive to mechanical stimulation. Both ex vivo passive stretch (i.e., stretch performed on a muscle removed from the body) and in vivo eccentric actions (i.e., actions of a muscle that is intact in the body) were found to increase PA and mTOR signaling (91). Moreover, administration of 1-butanol—a PLD antagonist—blunts both PA synthesis and mTOR signaling (114). In combination, these data indicate that PLD-derived PA is integrally involved in the mechanical activation of mTOR (91). It should be noted that PA can be synthesized by alternative enzymes, and there is some evidence that its activation by diacylglycerol kinase may play a role in its hypertrophic effects as well.

AMPK Pathway

The trimeric enzyme 5′-AMP-activated protein kinase (AMPK) plays a key role in the regulation of cellular energy homeostasis. AMPK acts as a cellular energy sensor; its activation is stimulated by an increase in the AMP/ATP ratio (90). As such, conditions that elicit substantial intracellular energy stress—including exercise—can activate AMPK. Once activated, AMPK suppresses energy-intensive anabolic processes such as protein synthesis and amplifies catabolic processes, including protein breakdown (90).

Because of its inherent actions, AMPK is theorized to be involved in the maintenance of skeletal muscle mass. This contention is supported by evidence showing that knockout (inactivation) of AMPK in animal models causes hypertrophy both in vitro and in vivo (90). Alternatively, activation of AMPK by AICAR—an AMPK agonist—promotes myotube atrophy, whereas its suppression counteracts the atrophic response (90). Taken together, these findings indicate that AMPK impairs muscle hypertrophy by suppressing protein synthesis and stimulating proteolysis.

The precise mechanisms by which AMPK carries out its actions are still being elucidated. Proteolytic effects of AMPK appear to be related at least in part to its influence over atrogin-1. Protein degradation induced by AMPK agonists (AICAR and metformin) has been found to correlate with atrogin-1 expression, whereas another AMPK antagonist (Compound C) blocks such expression. Evidence shows that these actions may involve an AMPK-induced increase in FOXO transcription factors, thereby stimulating myofibrillar protein degradation via atrogin-1 expression (194). AMPK has also been shown to induce protein degradation via activation of *autophagy* (regulated cell degradation by organelles termed lysosomes) (90), although it remains to be determined whether this mechanism plays a role in skeletal muscle adaptations following mechanical overload. Other research indicates that AMPK reduces cell differentiation of myoblasts and thus negatively affects hypertrophic adaptations without necessarily accelerating protein degradation (286).

In addition to the catabolic actions of AMPK, compelling evidence suggests that it suppresses the rate of protein synthesis. It is theorized that this negative influence is mediated at least in part by antagonizing the anabolic effects of mTOR, either by direct phosphorylation of mTOR, indirect phosphorylation of the tuberous sclerosis complex (TSC), or both, which has the effect of inhibiting the Ras homolog enriched in brain (RHEB) (187, 258). The upshot is an inhibition of translation initiation (19), the rate-limiting step in muscle protein synthesis.

Another potential means whereby AMPK is theorized to negatively affect muscle protein synthesis is the inhibition of translation elongation and the indirect suppression of the anabolic effector eIF3F (90). Thus, there are

multiple potential mechanisms for AMPK-mediated regulation of protein synthesis.

A number of studies lend support to the theory that AMPK plays a role in the muscular adaptations in response to regimented exercise training. AMPK activation shows a strong inverse correlation with the magnitude of muscle hypertrophy following chronic overload (275). In addition, AMPK inhibition is associated with an accelerated growth response to mechanical overload, whereas its activation attenuates hypertrophy (90). However, other research calls into question the extent to which AMPK regulates exercise-induced hypertrophy. In humans, mTOR signaling and muscle protein synthetic rate are elevated following resistance exercise despite concomitant activation of AMPK (54). This indicates that, at the very least, the activation of AMPK is not sufficient to completely blunt growth. Moreover, growth in mice lacking the primary upstream kinase for AMPK was not enhanced following functional overload, casting uncertainty about the importance of AMPK in muscular adaptations to mechanical loading (175).

MSTN-SMAD Pathway

The role of MSTN in muscle hypertrophy was outlined in chapter 1 and thus will only be briefly discussed here. MSTN, a member of the transforming growth factor-β superfamily, is a potent negative regulator of muscle growth. Knockout of the MSTN gene causes hypermuscularity whereas its overexpression causes atrophy. MSTN carries out its effects through activation of SMAD2 and SMAD3 (via phosphorylation of activin Type I receptors), which in turn translocate to the cell nucleus and regulate transcription of target genes via interaction with DNA and other nuclear factors (229).

MSTN plays an important role in the maintenance of muscle mass, and its expression decreases in almost all resistance exercise studies. Intriguingly, some research shows correlations between resistance training–induced reductions in MSTN and subsequent increases in muscle growth (222), while other research has failed to demonstrate such associations (66). The specific role of MSTN with respect to its hypertrophic effects during resistance training therefore remains to be fully elucidated. For further insights on the topic, please see the myokine section that covers MSTN in chapter 1.

Metabolic Stress

Although the importance of mechanical tension in promoting muscle growth is indisputable, there is evidence that other factors also play a role in the hypertrophic process. One such factor proposed to be of particular relevance to exercise-induced anabolism is *metabolic stress* (230, 244, 255). Simply stated, metabolic stress is an exercise-induced accumulation of metabolites, particularly lactate, inorganic phosphate, and H+ (261, 272). However, it should be noted that approximately 4,000 metabolites have been detected in human serum (294), and thus other metabolic byproducts may be relevant to training-related adaptations as well. Several researchers have surmised that metabolite buildup may have an even greater impact on muscle hypertrophy than high-force development (250), although other investigators dispute this assertion (71).

Metabolic stress is maximized during exercise that relies heavily on anaerobic glycolysis for energy production, which is characterized by a reduced PCr concentration, elevated lactate levels, and a low pH. Anaerobic glycolysis is dominant during exercise lasting 15 to 120 seconds, and corresponding metabolite accumulation causes peripherally (as opposed to centrally) induced fatigue (i.e., fatigue related to metabolic or biochemical changes, or both, as opposed to reductions in neural drive) (227). Research shows that performing 1 set of 12 repetitions to failure (with a total time under tension of 34 to 40 seconds) elevates muscle lactate levels to 91 mmol/kg (dry weight), and

KEY POINT

Evidence suggests that metabolic stress associated with resistance training can promote increases in muscle hypertrophy, although it is unclear whether these effects have a synergistic relationship with mechanical tension or are redundant.

values increase to 118 mmol/kg after 3 sets (160). In contrast, minimal metabolite buildup is seen in protocols involving very heavy loading (≥90% of 1RM) because the short training durations involved (generally <10 seconds per set) primarily tap the phosphagen system for energy provision. In addition, muscle oxygenation is compromised during resistance training that relies on fast glycolysis. The persistent compression of circulatory flow throughout a longer-duration set results in acute hypoxia, thereby heightening metabolite buildup (268). The combination of these factors causes the rapid accumulation of intramuscular metabolites along with a concomitant decrease in pH levels (260).

Typical bodybuilding routines are intended to capitalize on the growth-promoting effects of metabolic stress at the expense of higher intensities of load (77, 240). These routines, which involve performing multiple sets of 8 to 12 repetitions per set with relatively short interset rest intervals (145), have been found to increase metabolic stress to a greater degree than higher-intensity regimens typically employed by powerlifters (135-137). It is well documented that despite regular training at moderate intensities of load, bodybuilders display hypermuscular physiques and levels of lean body mass at least as great as, if not greater than, those achieved by powerlifters (77, 128). Indeed, there is evidence that bodybuilding-type routines produce superior hypertrophic increases compared to higher-load powerlifting-style routines (39, 171, 238), although findings are not consistent across all trials when equating for volume load (32, 243).

Evidence suggests that various metabolites can directly function as hypertrophic stimuli. Of particular relevance, several studies have demonstrated that culturing muscle cells in vitro with lactate enhances anabolic signaling and myogenesis (204, 205, 282, 303), and similar results have been shown using an in vivo murine model (34). An in vivo study by Oishi and colleagues (205) showed that daily oral administration of a lactate and caffeine supplement combined with treadmill exercise produced significantly greater hypertrophic increases in the gastrocnemius and tibialis anterior muscles of male rats than in the exercise-only and sedentary control groups. The inclusion of caffeine in the supplement confounds these findings, although acute results from the same study showed only minor benefits of the combination of lactate and caffeine versus lactate alone on markers of intracellular anabolic signaling. Similarly, Ohno and colleagues (204) found that oral lactate administration in mice increased fiber cross-sectional area of the tibialis anterior along with a corresponding increase in Pax7-positive nuclei in the muscle. Most recently, Tsukamoto and colleagues (282) demonstrated that intraperitoneal injection of lactate in rodents, at levels similar to those seen following resistive exercise, elicited significantly greater hypertrophy of the tibialis anterior muscle compared to saline injection. Although the mechanisms of potential hypertrophic effects of lactate have not been elucidated, it is hypothesized that they may be regulated via calcium-dependent signaling pathways (205). Notably, these animal studies do not replicate the in vivo response during human exercise, so the practical implications of results must be interpreted cautiously. Intriguingly, lactate production serves to inhibit activity of histone deacetylase (149), a negative regulator of muscle growth (179), thereby providing another potential avenue for inducing hypertrophic effects.

There also is evidence that H+ can alter hypertrophic adaptations. Type II fibers are particularly sensitive to acidosis. It is theorized that the intramuscular buildup of H+ impairs calcium binding in these fibers, causing a progressive reduction in their force-producing capacity as metabolically taxing exercise continues (97). Accordingly, this places an increased burden on Type I fibers to maintain force output and conceivably enhance their development. Several additional factors are theorized to mediate hypertrophic adaptations from exercise-induced metabolic stress, including increased fiber recruitment, myokine production alterations, cell swelling, metabolite accumulation, and elevated systemic hormone production (93, 94, 198, 265). What follows is a discussion of how these factors are thought to drive anabolism (figure 2.4).

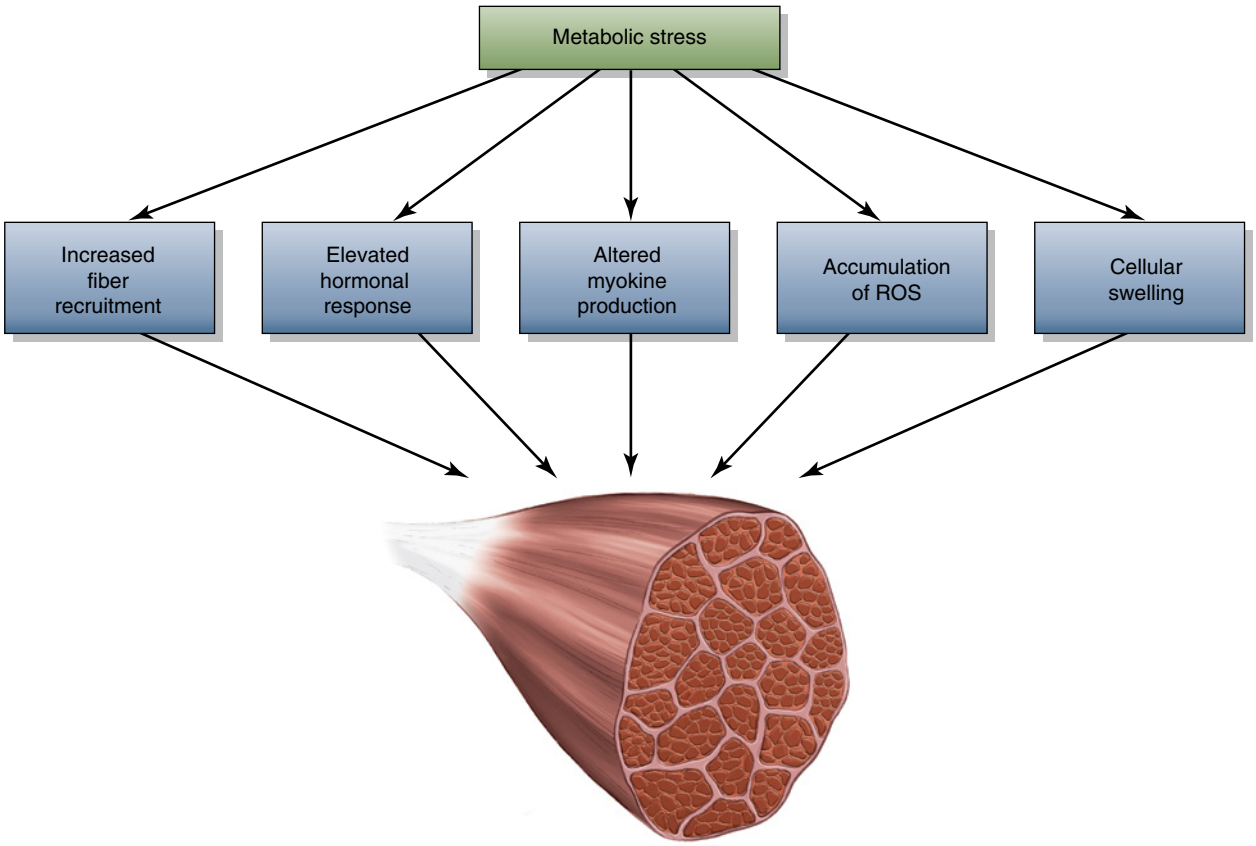

FIGURE 2.4 Mechanisms of metabolic stress.

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Fiber Recruitment

As discussed in chapter 1, muscle fiber recruitment is carried out in an orderly fashion whereby low-threshold motor units are recruited first and then higher-threshold motor units are progressively recruited thereafter to sustain muscle contraction depending on force demands (110). Although heavy loading rapidly activates the full spectrum of fiber types, research indicates that metabolic stress increases the recruitment of higher-threshold motor units even when lifting light loads. Studies show that as fatigue increases during sustained submaximal exercise, recruitment thresholds correspondingly decrease (116, 234, 293). Accordingly, activation of fast-twitch fibers is high provided a set is carried out to the point of muscular failure. Studies employing electromyography (EMG) (265, 266), glycogen depletion (123), and organic phosphate splitting (260, 261) have all demonstrated increased fast-twitch fiber activation in BFR training, causing some researchers to speculate that this is the primary factor by which occlusion mediates anabolism (155, 182).

The precise mechanisms whereby metabolic stress augments fast-twitch fiber recruitment are not entirely clear. It has been hypothesized that H+ accumulation plays a substantial role by inhibiting contractility in working fibers and thus promoting the recruitment of additional high-threshold motor units (51, 186, 266). MacDougall and colleagues (160) proposed that fatigue during single-set training to failure is due to a combination of acidosis and PCr depletion, whereas acidosis is more likely the cause in multiset resistance exercise.

Although it would seem that increased fiber recruitment is at least partly responsible for the

RESEARCH FINDINGS

BLOOD FLOW RESTRICTION

The impact of metabolic stress on hypertrophic adaptations is exemplified by *blood flow restriction* (BFR) training studies. BFR training involves restricting venous inflow via the use of a pressure cuff while training (figure 2.5) with light weights (generally equating to <40% of 1RM), thereby heightening ischemia in the muscle as it contracts.

The prevailing body of literature shows that BFR training stimulates anabolic signaling and muscle protein synthesis (78) and markedly increases muscle growth (156) despite employing loads often considered too low to promote significant hypertrophy (32, 140).

FIGURE 2.5 A blood flow restriction implement on an arm.

It has been speculated that metabolic stress is the driving force behind BFR-induced muscle hypertrophy. Significant metabolite buildup has been noted during such training (154), pointing to an association between metabolic stress and muscle growth. In further support of this contention, significant increases in cross-sectional area of the thigh muscle were found in college-aged males after 3 weeks of walking with BFR of the legs (3). Given that healthy young subjects generally do not gain muscle from performing low-intensity aerobic exercise, the study provides strong evidence that factors other than mechanical tension were responsible for hypertrophic adaptations. Indeed, increases in muscle cross-sectional area were found to be significantly correlated with the changes in inorganic phosphate (r = .876) and intramuscular pH (r = .601) during BFR training carried out at 20% of 1RM. Results suggest that metabolic stress generated during exercise may be a key regulator of muscle growth (263). In further support of this hypothesis, evidence indicates that ischemia—a potent mediator of metabolic stress—upregulates anabolic myokines during exercise performance, although the duration of these elevations is rather transient, returning to baseline values within approximately 30 minutes after cessation of training (249).

Studies investigating resistance training under conditions of hypoxia provide further evidence of an association between metabolic stress and muscle growth. Kon and colleagues (133) found that breathing 13% oxygen during a multiset, low-load (~50% of 1RM) protocol with fairly short interset rest intervals (~1 minute) significantly heightened blood lactate levels compared to the same routine performed under normoxic conditions, providing proof of principle that hypoxia heightens metabolite accumulation. Several studies show that hypoxia enhances the hypertrophic response to resistance training. Nishimura and colleagues (198) reported significantly greater increases in elbow flexor cross-sectional area when 4 sets of 10 repetitions at 70% of 1RM were performed under conditions of acute hypoxia versus normoxia. Similar findings have been reported elsewhere (19). Although these findings do not provide a causal link between hypertrophy and metabolic stress, they raise the possibility that increased metabolite accumulation plays a role in the process (247). It should be noted that reactive oxygen species (ROS) released during conditions of hypoxia have been implicated in carrying out a variety of signaling responses, providing another

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