brief review

Skeletal muscle fiber hyperplasia

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ABSTRACT

ANTONIO, J. A. and W. J. GONYEA. Skeletal muscle fiber hyperplasia. Med. Sci. Sports Exerc. Vol. 25, No. 12, pp. 1333–1345, 1993. Skeletal muscle enlargement in adult animals has been ascribed primarily to changes in fiber cross-sectional area (i.e., fiber hypertrophy); however, recent evidence from several laboratories suggests strongly that fiber hyperplasia contributes to muscle mass increases in adult animals and possibly human athletes. Scientists have used three models to study the cellular mechanisms of muscle enlargement: compensatory hypertrophy, stretch, and exercise. Each of these models has provided direct as well as indirect evidence supporting the occurrence of muscle fiber hyperplasia. Direct counts of muscle fibers using nitric acid digestion techniques have shown that both exercise and stretch overload result in significant increases (range = 9–52%) in fiber number. Indirect fiber counts using histological cross-sections have suggested fiber hyperplasia (range = 10–82%) in all three models. Additionally, the expression of embryonic myosin isoforms have provided indirect evidence for new fiber formation in stretch overloaded muscle. Furthermore, satellite cells have been shown to be involved in muscle fiber hyperplasia in stretch and exercise.

EXERCISE, HYPERTROPHY, STRETCH

Skeletal muscle undergoes profound morphological, biochemical, and physiological changes as a result of increased contractile activity. The adaptations that muscle fibers undergo are directly related to the stimulus presented. Endurance exercise training, which involves prolonged muscular contractions designed to increase VO_{2max} (i.e., the maximum amount of oxygen transferred to circulation per minute) and muscle oxidative capacity, results in increments in cardiac output, mitochondrial volume density, myoglobin, and Krebs cycle enzymes (21,40,49). On the other hand, heavy resistance training produces effects different from endurance training. An increase in fiber area (hypertrophy) and fiber number (hyperplasia) contribute to the overall increase in muscle girth (33,35). Moreover, mitochondrial volume density may actually decrease (46).

In addition to exercise models, other types of stimuli have been used to induce muscle enlargement (e.g., synergist ablation, stretch). Each of these models of muscle enlargement share a common feature in that the muscle(s) studied are subjected to an increased demand and that the muscle responds with an increase in muscle mass. The following review will not emphasize the advantages or disadvantages for each of these models (for review, 69); however, we will summarize evidence from these different models that provides convincing evidence that skeletal muscle fiber hyperplasia occurs in adult muscle.

Techniques for measuring muscle fiber number.

The two most common techniques for measuring muscle fiber number are through the use of histological cross-sections and after nitric acid digestion of the muscle to be examined (3–5,7,32). Histological counts are often obtained at the mid-belly of the muscle, which is generally the area of widest girth. Using myosin ATPase staining, it is relatively easy to obtain total fiber number and the percentage of each fiber type. However, certain drawbacks exist with regard to histological counts. Do all fibers run from origin to insertion? Does the cross-section of muscle examined contain every fiber within that muscle? The ALD muscle is a parallel fibered muscle in which all fibers in control and chronically stretched muscle extend from origin to insertion (4). However, in ALD muscle that undergoes progressive, intermittent stretch overload, new fibers are formed that do not run from origin to insertion (unpublished observations). So even a small, parallel fibered muscle such as the ALD may present problems in obtaining accurate counts. In muscles with a pennate arrangement, it becomes more difficult to obtain accurate counts due to the changes in fiber angle as a result of muscle hypertrophy (31). Consequently, scientists have used nitric acid digestion of muscle as the definitive method of obtaining total fiber number (3,5,32).

Nitric acid digestion of muscle makes it possible to tease each fiber individually and count each fiber sep-
arately as well as those with bifurcations. This method, although the most direct method of counting fibers, has its drawbacks. It is not unusual to obtain histological counts that are greater than direct counts (i.e., after nitric acid digestion) (4,8,9). Small fibers may be missed when performing direct counts and may result in an underestimation of the total fiber number. Nevertheless, direct counting after nitric acid digestion is the most widely accepted technique for fiber enumeration.

In vivo estimates of fiber number can be obtained using the following formula: Total muscle fiber number = muscle cross-sectional area (corrected for noncontractile tissue)/mean fiber area (6). Mean fiber area is obtained from tissue derived from a needle biopsy. One must assume that the muscle biopsy sample is representative of the entire muscle. Since muscle is not a homogenous tissue, this assumption may contribute to sampling error especially if only one biopsy is used. Also, the biopsied tissue undergoes a postexcision contraction that would consequently affect the cross-sectional area of the examined fibers. The result would be an overestimation of fiber area with a resultant underestimation of fiber number (6). To account for this postexcision contraction, MacDougall et al. (48) corrected fiber number by 36%. That is, the biceps brachii muscle cross-sectional area is 36% greater when the elbow is in a flexed vs extended position. Therefore, the actual number of muscle fibers exceeds the estimated number by approximately 36%, assuming that fiber volume is constant.

Postnatal growth. It is commonly accepted that fiber number increases during fetal growth and that any subsequent growth postnatally is due primarily to fiber hypertrophy (31,32). However, there is evidence to suggest that both fiber hypertrophy and hyperplasia contribute to muscle growth during fetal and early postnatal life in humans and animals (1,19,54,60). Montgomery (54) examined human sartorius, a parallel-fibered muscle, and found that fiber number from histological cross-sections doubled from the 32nd week of fetal life to 4 months postnatal (64,000 fibers in the 32-wk-old fetus to 134,000 fibers in a 4 month old). It is also noteworthy that there were no differences in fiber number in sections taken at the proximal, middle, and distal regions of the sartorius muscle. Therefore, the fiber number increase would not have been due to the growth of existing fibers into the area of muscle that was examined in cross-section.

Studies on muscle growth in rats demonstrate similar patterns as in the human. Chaikulus and Pauly (19) studied the soleus, plantaris, and extensor carpi radialis longus muscles of rats 1, 3, 6, 12, and 24 wk of age. Fiber counts were derived from a transverse section through the thickest portion of the muscle belly. The cross-sectional area of the muscle increased approximately 1000% in these muscles between 1 and 24 wk while fiber number increased approximately 60%. Rayne and Crawford (60) had similar findings in the rat pterygoid muscle studied postnatally. The pterygoid muscle was chosen because it is a small muscle (making fiber counts feasible), and the fibers run from origin to insertion. In the male rats, the fiber number in the medial pterygoid muscle increased 100% between birth and 6 wk while the lateral pterygoid muscle increased approximately 45% between birth and adult.

Muscle Hypertrophy in Adult Animals

Compensatory hypertrophy—synergist ablation and tenotomy. Denny-Brown (26) was the first to use the compensatory hypertrophy model. This model involves the severing of a tendon or complete removal of a muscle. The synergist muscles are consequently subjected to an increase in functional demand resulting in muscle hypertrophy. The compensatory hypertrophy model is the most often used model to study muscle enlargement.

Armstrong et al. (10) examined the acute response of the plantaris muscle to removal of the gastrocnemius muscle. They found that the initial wet weight increase (+14%) in the plantaris muscle was due largely to inflammation resulting in edema and leukocyte infiltration. They hypothesized that the cause of this inflammation was due largely to surgical trauma. Snow (63) found that fiber cross-sectional area initially decreases 3 d post surgery in the overloaded soleus muscle due to the initial stretch of the soleus muscle fibers caused by ankle dorsiflexion. However, after the initial inflammatory response, muscle fiber cross-sectional area increases 10–40% 1–2 months post surgery (63,71).

Gollnick et al. (31) examined the effect of synergist ablation and exercise on muscle fiber number in the soleus, plantaris, and extensor digitorum longus muscles of rats. They found no differences in muscle fiber number for any of the muscles examined. These investigators concluded that muscle fiber hypertrophy can fully account for the observed muscle mass increase.

Vaughn and Goldspink (71) tenomedized the gastrocnemius and plantaris muscle of young mice and examined the adaptive response of the soleus muscle. Two-hundred-eight days (~7 months) post surgery, soleus wet weight was 46% greater in the tenomized animals. Mean fiber diameter was 5% smaller in the distal region but 10% larger in the proximal region of the overloaded soleus muscle when compared with the control. In addition, fiber number was 33% and 10% greater in the distal and proximal regions of the overloaded soleus muscle versus the control. The reasons for regional differences in fiber number and area are not apparent although regional fiber area differences have been reported in stretch-induced enlargement (4). Vaughn and Goldspink (71) speculated that the increase in fiber number in the distal region of the muscle was
due to fiber splitting. Electron microscopic observations of splitting fibers supported this; however, they did not quantify the number of split fibers. Thus, the contribution of fiber splitting to the observed fiber number increase is not clear. Regardless, the fact that muscle mass increased 46% and mean fiber diameter increased at most 10% suggests strongly that increases in fiber area cannot totally account for the muscle mass increase (i.e., fiber number and/or fiber length changes must account for the rest of the mass increase). Muscle mass can be predicted from the formula: (fiber cross-sectional area × fiber number × fiber length × muscle density) = muscle mass. Since muscle density does not change with overload, changes in fiber length, fiber cross-sectional area, and/or fiber number must account for the muscle mass increase.

Timmson et al. (70) investigated compensatory hypertrophy in the soleus muscle 6–8 wk after removal of the plantaris and gastrocnemius muscles. These investigators found that mean fiber area increased nearly 50% in male mice; moreover, increases in muscle mass could totally be accounted for by increases in fiber area. Fiber counts using nitric acid digestion methods showed no differences between control and enlarged muscle. Chalmers et al. (18) examined the response of the plantaris muscle after excision of the gastrocnemius muscle in adult cats. Approximately 4 wk postsurgery, these cats were started on an exercise program that consisted of walking around the room for up to 15 min·d⁻¹, 6 d·wk⁻¹. Exercise intensity was increased by throwing tennis balls around the room to elicit jumping and running activities in the cats. Twelve weeks postsurgery, muscle mass was approximately 136% greater in the overloaded plantaris muscle vs the control. Mean fiber cross-sectional area was 125% and 172% larger in the light and dark ATPase fibers (alkaline preincubation) of the overloaded plantaris muscle. It would seem that the 172% increase in the dark fibers is an overestimation since fiber cross-sectional area increases should not exceed muscle mass increases (unless fiber number and/or fiber length is decreasing). Less than 100 fibers were examined in the plantaris muscle, and this small sample may not truly reflect the mean fiber cross-sectional area of the muscle. Nevertheless, these investigators also reported the presence of very small fibers in some of their cats, which suggests the possibility of fiber hyperplasia.

Small fibers have also been seen in cats (29) and rats (38) after resistance training and in the rat plantaris after surgical ablation (77). Yamada et al. (77) have shown that some of the small fibers observed in overloaded plantaris muscle following synergist ablation express embryonic and/or neonatal myosin. These investigators suggested that this small fiber population in the hypertrophied plantaris muscle was formed de novo and did not arise by fiber splitting.

**Stretch.** An additional model for studying the mechanisms of muscle enlargement involve imposing a stretch on a particular muscle (64) (Fig. 1). Sola et al. (64) demonstrated that the anterior latissimus dorsi (ALD) muscle in the adult chicken underwent significant enlargement in response to a chronic stretch with weight. With 200 g attached to the left wing for 6–8 wk (and the right side serving as the intra-animal control), muscle wet weight increased 120% and 180% in denervated and innervated muscle relative to the intra-animal control. Although the innervated muscle responded the greatest to stretch, significant enlargement occurred in the denervated muscle, indicating that neural input is not necessary for muscle enlargement. However, it could be speculated that there may exist an active component (i.e., prolonged isometric contraction) in the stretch model; otherwise, the response between the denervated and innervated muscle would be similar. Fiber numbers were obtained via counts of histological cross-sections and demonstrated that both fiber hypertrophy (~130% increase) and hyperplasia (~16% increase) contributed to the muscle mass gain.

Other investigators have not duplicated Sola and coworkers’ results showing increased fiber number.

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**Figure 1—Avian stretch model.** A Velcro cut filled with lead pellets is wrapped around one wing of the bird while the opposite wing serves as the control. The muscle most often studied is the ALD. Other muscles studied include the patagialis and biceps brachii. Stretch enlarged ALD muscle has shown consistent and predictable fiber hyperplasia (up to an 80% increase in fiber number) (3–5,8,9).
Holly et al. (39) also looked at chicken wing muscles using a spring-loaded tubular assembly as a method of inducing stretch. They found that longitudinal growth in the patagialis muscle was completed within 1 wk whereas cross-sectional area increased throughout the 5-wk stretch period. In a parallel study done by Barnett et al. (13), they found no evidence of fiber hyperplasia. In the patagialis muscle, increases in mass were accounted for by increases in fiber cross-sectional area. Ashmore and Summers (11) confirmed the earlier work of Holly et al. (39) and Barnett et al. (13). Observations of increased fiber number in Sola et al. (64) vs no change in the previously cited reports could be due in part to the different muscles examined.

The patagialis muscle is a twitch muscle whereas the ALD muscle is a tonic muscle and differs from the former in that it has many motor end plates that receive branches from more than one neuron (polynuclear innervation). However, it has been shown in exercising animals (35, 38, 66) that fiber hyperplasia can occur in twitch muscles. Also, the method of stretch used by Holly et al. (39) involved using a spring-loaded device consisting of two hollow tubes with an internal spring. These were attached to the humerus and ulna through pins inserted into the bone. Obviously, this differs from the method Sola et al. used (i.e., wrapping a weight around the wing) and therefore makes it difficult to compare the results from these different stretch models.

Gollnick et al. (32) examined the ALD muscle in the adult chicken. Using the method of Sola et al. (64), they found that muscle that was weighted for a period ranging from 6–65 d weighed an average of 105% greater than control muscles. However, using both histological counts and direct counts, they found no increase in fiber number. It is difficult to discern why these results differed from those of Sola et al. (64). The mass of the birds used in this study were not reported; consequently, the weight used to stretch the birds’ muscle may not have been heavy enough to induce fiber hyperplasia.

Nevertheless, recent work from independent laboratories have strongly suggested that fiber hyperplasia may contribute to stretch-induced muscle hypertrophy (3, 5, 8, 9, 42, 51). Kennedy et al. (42) found that small fibers in the stretched ALD muscle contained an embryonic form of myosin. Furthermore, these small fibers were found mainly outside the boundaries of the fascicles and were not part of injured muscle fibers. These lines of evidence, albeit qualitative, suggest that fiber hyperplasia contributed to the muscle mass increase.

A morphological study by Alway et al. (4) further showed that stretch-induced enlargement of the quail ALD muscle was due both to fiber hypertrophy and hyperplasia. These investigators stretched the ALD muscle of adult quails for 30 d with a weight corresponding to 10% of the bird’s body weight. This produced an average of 172% increase in muscle mass and a 23.5% increase in muscle length. Mean fiber cross-sectional area increased by 57%; in addition, fiber number increased 52% in the stretched muscle. This was confirmed using histological counts and direct counts using nitric acid. Interestingly, fiber number was significantly greater by an average of 41% in the stretched ALD muscle 60 d after the removal of the stretch, yet fiber cross-sectional area had returned to control levels (3). This would suggest that the mechanisms responsible for downregulating fiber area and number are different in the quail ALD muscle.

Additionally, these investigators (5) examined the morphologic response during the first week of chronic stretch. They found that muscle mass increased significantly after 2 d of stretch; however, this was accounted for by a nonproportional increase in connective tissue. Fiber number increased significantly by day 5, yet fiber area did not increase significantly until day 7. This suggests that fiber hypertrophy does not precede fiber hyperplasia in the chronic stretch model. Using the same stretch protocol as Alway and colleagues (4), McCormick and Schultz (51) found that 1 wk of stretch resulted in the formation of numerous small fibers. These small fibers expressed a ventricular-like embryonic myosin. Additionally, the majority of these fibers were located between fascicular spaces and had an elevated nuclear density. These investigators suggested that de novo fiber formation is the principal mechanism by which fiber hyperplasia occurs.

In contrast to chronic stretch, intermittent stretch of the ALD muscle produces muscle fiber hypertrophy without hyperplasia (7). The muscle fibers of intermittently stretched muscle were all found to be infr fascicular and muscle mass increases could be accounted for by changes in muscle length and fiber cross-sectional area. Antonio and Gonyea (8) further examined the adaptive response of the ALD muscle undergoing progressive stretch overload. In this model, it is possible to separate the components of muscle fiber hypertrophy and hyperplasia (Figs. 2 and 3). Weights ranging from 10–35% of the bird’s mass were wrapped around the right wing of adult quail while the left wing served as the intra-animal control. Each increment in weight was interspersed with a 2- to 3-d rest interval. In the progressive stretch overload model, it was observed that significant myofiber hypertrophy occurs prior to fiber hyperplasia. The initial peak in mean fiber cross-sectional area (~142% increase) for the stretched ALD muscle is followed by a subsequent decline in mean fiber cross-sectional area. Fiber hyperplasia (~82% fiber number increase) coincided with the decline in mean fiber cross-sectional area and the appearance of small fibers.

In addition, these investigators observed the presence of large fibers with fissures or clefts extending into the
interior of the fiber, suggesting the possibility that the splitting of these large fibers may contribute to fiber hyperplasia (Fig. 4). In fact, the incidence of branched or splitting fibers in ALD muscle (i.e., ~4–5% of the total fibers exhibit branches or splits) undergoing progressive stretch overload exceed any previous report of adult skeletal muscle enlargement (9). Alternatively, these large fibers with fissures may undergo a degenerative response and cause satellite cell proliferation.

**Exercising animals.** Several animal models (e.g., cats, rodents) exist that resemble resistance training performed by humans. Gonyea and Ericson (34) developed a resistance training model using cats (Fig. 5). Cats were trained to perform wrist flexion against progressively increased resistance in order to receive a food reward. This model was good in that it involved voluntary exercise and the unexercised contralateral limb could serve as an intra-animal control. Gonyea et al. (35) demonstrated that 101 wk of progressive resistance training resulted in a 11% and 9% increase in muscle mass and fiber number of the flexor carpi radialis muscle, respectively. Fiber numbers were obtained via direct counts using the nitric acid digestion technique.

Mikesky et al. (53) used the cat weight lifting model to examine the response of the palmaris longus muscle (PLM). After an average of 150 wk of training, the exercised PLM demonstrated a 24% increase in muscle mass while fiber cross-sectional area increased 11%. There was no change in fiber length or volume density of connective tissue; therefore, the observed increase in muscle fiber cross-sectional area could not totally account for the observed muscle mass increase. This would suggest that fiber hyperplasia contributes in part to the muscle mass increase. Also, Giddings and Gonyea (30) observed small fibers in the exercised PLM of cats, which would suggest de novo fiber formation.

Although this model most closely mimics human weight lifting, its inability to make predictable gains in muscle mass make it unfeasible to study the cellular
mechanisms of muscle enlargement. However, Mikesky et al. (52) examined the various training variables which were the best predictors of muscle hypertrophy. They found that the training variable demonstrating the highest correlation to changes in muscle mass was the average lift time (r = 0.71). That is, they found that the cats which lifted the weight in a slow and deliberate manner made greater mass gains than cats which lifted the weights in a ballistic manner. Perhaps future studies using the cat weight lifting model should control for speed of movement in order to elicit a better and more predictable muscle mass increase.

Other models involving rodents have employed the attachment of weights to the tail or trunk of the animals' body and having the animal stand upright or climb a certain distance vertically (38, 78). Ho et al. (38) examined male rats after 8 wk of weight lifting exercise and found a significant increase in the adductor longus muscle weight. Total muscle cross-sectional area was greater in the weight lifting group; however, the fiber cross-sectional areas of the slow oxidative and fast oxidative-glycolytic fibers were smaller in the weight lifting group. Moreover, the number of fibers per unit cross-sectional area was greater in the weight lifting rats. Ho and coworkers suggested that muscle fiber splitting accounted for the increase in fiber number even though split fibers were not quantified.

Tamaki et al. (66) examined the adaptive response of the plantar flexor muscles to sprint training and weight lifting (Fig. 6) and compared it with untrained sedentary rats. The weight lifting group performed 15 sets of 15 repetitions of squat exercises. The repetitions were performed every 2 s with a 2-min rest interval between sets. Every 2 wk the load was readjusted to accommodate the increased strength of the rats. The training period lasted 12 wk. Using nitric acid digestion of muscle, total fiber number in the plantaris muscle was significantly greater in the squats group (14% higher) vs both the sprint-trained and sedentary group. These investigators also reported an increase in the absolute number of branched fibers in the squats group; however, closer examination of the data reveals that branched fibers occur in less than 0.5% of the fibers in the enlarged plantaris muscle. Thus, the contribution of fiber branching to fiber hyperplasia is negligible in this weight lifting paradigm, or fiber branching (i.e., splitting) may occur so rapidly as to go undetected.
Yarasheski et al. (78) trained rats to carry a progressively heavier load while climbing a mesh basket at a 90° angle in order to receive a food reward. They found that total cross-sectional area of the trained rectus femoris muscle was 6.4% greater than the muscles from sedentary rats. However, fiber numbers derived from histological cross-sections were similar between trained and untrained rats. It is unclear why the weight lifting rats in Yarasheski et al.'s study (78) did not increase fiber number; however, the extremely modest increases in muscle cross-sectional area that the weight lifting rats accrued would suggest that training duration and/or intensity was insufficient. Or conversely, these rats may have been overtrained due to the frequency of training (five training sessions per week). Would less frequent training have produced greater mass gains?

Animal exercise models have yielded confusing results due to the differences in training protocol, species of animal used, and methods of estimating fiber number. However, evidence in weight lifting cats (35) and rats (66) strongly indicate that resistance training can increase fiber number.

**Exercising humans.** Experiments using human subjects have consistently shown that heavy resistance training is the best way of producing muscle hypertrophy (12,14,20,27). In general, long-term heavy-resistance training produces increases in muscle mass with this increase due primarily to increased fiber cross-sectional area (36,59,61); however, increases in fiber number can not be ruled out (43,55,67). The increase in muscle cross-sectional area is due primarily to a preferential hypertrophy of Type II fibers (22,45,59).

Furthermore, there is a decrease in mitochondrial volume (46) and capillary density (68).

Several researchers have speculated that there may be a maximal size that muscle fibers attain as a result of heavy-resistance training (47,67). Despite the fact that elite bodybuilders and powerlifters have arm circumferences 26% greater than normal sedentary controls, there were no differences in fiber areas for either slow-twitch or fast-twitch muscle in the triceps brachii muscle (47). Additionally, there were no differences in mean fiber area in the deltoid and vastus lateralis muscles in bodybuilders vs. physical education students (67). MacDougall et al. (48) further examined the contribution of fiber number in the biceps brachii of bodybuilders and sedentary subjects. They found that bodybuilders had significantly greater fiber cross-sectional areas compared with sedentary subjects; however, both groups possessed the same number of fibers in the biceps brachii muscle. Although some of the bodybuilders possessed a higher than average fiber number, there were a few sedentary subjects who had similar fiber numbers.

MacDougall et al. (48) suggested that the massive arm size of bodybuilders is due primarily to larger fiber cross-sectional areas but also to genetic endowment of superior fiber numbers. Hence, the bodybuilder who is born with more fibers has a greater potential for achieving the greatest gains in muscle mass. The implausibility of this argument can be seen when examining their previous data (47). If indeed these bodybuilders (47) inherited a greater number of fibers in their triceps brachii, that would suggest that it took 7 yr of intense

Figure 6—Rat weight lifting model developed by Tamaki et al. (66). Rats were fastened to a wooden arm via a canvas jacket with the resistance or weight placed on the wooden arm. Rats were conditioned to perform a squat movement through the use of electrical stimulation given to the tail. As a result of the electrical stimulation, the rats extended their legs and hips, which would lift the weight attached to the wooden arm. A safety stopper was set to prevent hyperflexion of the knee and ankle while a resting stopper was used to relax the legs during periods of rest. The plantaris muscle of rats trained in this fashion has shown modest fiber hyperplasia (14% increase in fiber number) (66).

![Rat weight lifting model](image-url)
training for these athletes to attain fiber sizes similar to previously sedentary subjects who trained for a mere six months! This would indicate that some bodybuilders are born with significantly greater number of small fibers. Alway et al. (6) compared the biceps brachii muscle in elite male and female bodybuilders. They found that most of the gender difference in muscle cross-sectional area was due to the greater mean fiber areas of the male bodybuilders; additionally, these male bodybuilders had a significantly greater number of small Type I fibers. Furthermore, it was shown that biceps muscle cross-sectional area was correlated to both fiber area and fiber number.

The greatest difficulty in determining the contribution of fiber size and number to hypertrophied human muscles are the technical and ethical constraints involved in the study of humans (i.e., it is unethical to remove whole muscles from living humans in order to get direct counts of fibers). Also, muscle biopsies can only give a limited picture of the muscle since it represents a very small portion of the muscle. A study by Sjöström et al. (62) demonstrated the difficulties in detecting fiber hyperplasia in human muscles. These investigators analyzed tibialis anterior (TA) muscles from the left and right legs of seven previously healthy right-handed men (mean age 23 yr, range 18–32) who experienced a sudden accidental death. It is known that in right-handed individuals, the musculature of the left leg tends to be larger and stronger due to asymmetrical use. Hence, their study examined the differences in fiber area and number between the right and left TA muscle. They found no differences in mean fiber area; however, the left TA muscle exhibited significantly greater muscle cross-sectional areas. This was due to differences in fiber number. The left TA muscle had 10% more fibers than the right TA (average fiber number left TA, 168,500; right TA, 153,100). This increase in fiber number was likely due to differences in activity patterns between the left and right TA muscle. These investigators proposed that if one assumes that it took approximately 10 yr to attain a difference of 15,000 fibers, that would mean that 30 new fibers are formed each week. With approximately 150,000 fibers in the TA, only 1 of 5000 fibers would be a new fiber formed in a given week. In a muscle biopsy comprising approximately 500 fibers, it becomes obvious how difficult it would be to detect a single new fiber (i.e., at least 10 biopsies would be needed to detect a single new fiber).

**Role of satellite cells in enlarging muscle.** Satellite cells were first reported by Mauro (50). It has been shown that it is the satellite cell which is the myogenic stem cell involved in skeletal muscle regeneration (16). Because skeletal muscle undergoes a degenerative response as a result of excessive tension and/or trauma, it seems plausible that there must exist a mechanism whereby this tissue can regenerate itself (2,16,17). Muscle regeneration begins following an injury or insult to a muscle fiber(s). This injury is characterized by myofibrillar disruption, mitochondrial abnormalities, sarcomemal discontinuity, and so forth. Satellite cells are then activated and a cell-mediated breakdown and removal of all traces of the originally injured muscle fiber(s) occur. Macrophages, as well as neutrophils and other cells, invade the interstitium. But in order for this to result, an intact functioning circulation is needed. During the peak phase of this cellular infiltration, numerous macrophages are present beneath the basal lamina of the damaged fiber, and these cells engulf the cytoplasmic debris. Hence, removal of the old muscle fiber is completed with an intact basal lamina remaining. Satellite cells then give rise to new myoblastic cells. The old basal lamina likely provides a building block or scaffold for the regenerating muscle cell (72).

**Activation of satellite cells.** Satellite cells are postulated to be the sole source of myonuclei added during postnatal growth (24). Consequently, factors that may affect growth of skeletal muscle will also influence the behavior of satellite cells. Exercise, ischemic injury, stretch, and transplantation have been shown to activate satellite cells (24,30,41,58,73,74). Darr and Schultz (24) observed the effects of eccentric treadmill running on satellite cell activation in the soleus (SOL) and extensor digitorum longus (EDL) muscles of 1- and 3-month-old rats. After a single bout of eccentric treadmill running (18% decline, 105 min), satellite cell activation was examined 24, 48, 72, and 120 h postexercise. Satellite cell activation increased in 1-month-old SOL and EDL muscles 72 h postexercise (~250% of control). Additionally, 3-month-old SOL muscles exhibited increased satellite cell activation 24 h postexercise (~250% of control). Interestingly, the amount of activated satellite cells exceeded that required to repair the number of injured (<3.0%) fibers identified at the light microscopic level, thus suggesting that fiber injury as well as other factors (e.g., tension) may activate satellite cells. Conversely, if muscle fiber tension activates satellite cells, the lack of tension or a decrease in normal tension should inhibit the activation of satellite cells. Darr and Schultz (25) demonstrated that hindlimb suspension, which is an atrophy model that mimics the effects of zero gravitational force, inhibited satellite cell activation in both SOL and EDL muscles.

Winchester et al. (73) demonstrated that chronic stretch of the anterior latissimus dorsi muscle of adult quail produced an increase in satellite cell activity during the first 3 wk of stretch relative to the control ALD muscle. These investigators suggested that the proliferation of satellite cells may be related to stretch-induced myofiber injury or the maintenance of a constant nuclear-to-cytoplasmic ratio in enlarged fibers of the
Muscle fiber hyperplasia (74). Furthermore, these investigators observed a temporal correlation between myofiber injury (74) and muscle fiber hyperplasia (5).

Bischoff (15) further examined the role of injury in the activation of satellite cells using an in vitro model. Single isolated muscle fibers and attached satellite cells were examined in culture and the response of satellite cells to a mitogen from crushed rat muscle were examined under three conditions: satellite cells in contact with the muscle fiber and its basal lamina, satellite cells physically separate from the muscle fiber, and satellite cells beneath the basal lamina in contact with a Marccaine killed myofiber. Satellite cells associated with killed muscle fibers were found to have a 40% greater activity in comparison to those satellite cells associated with viable muscle fibers.

Muscle hypertrophy—role of injury and satellite cells. Certainly, there is indirect evidence supporting the role of myofiber injury and therefore satellite cell proliferation in exercise-induced muscle hypertrophy. It has been well established that eccentric contractions produces greater injury to muscle than concentric contractions (28). Additionally, exercise training studies have shown that eccentric contractions are essential if muscle hypertrophy is to occur (23,37,75,76). Cote et al. (23) trained subjects for 10 wk using an isokinetic resistance training protocol (i.e., concentric work only). They found that despite a 54% increase in quadriceps femoris muscle strength, there was no change in muscle fiber cross-sectional areas. Hather et al. (37) compared concentric/eccentric training with concentric/concentric training using leg press and leg extension exercises twice a week for 19 wk. Only the concentric/eccentric group increased Type I fiber area whereas both groups increased Type II fiber area. Interestingly, 4 wk of detraining resulted in a decrement in fiber cross-sectional area for the concentric/concentric group. However, the concentric/eccentric group maintained hypertrophied Type I fibers even after a month of detraining.

In a rat weight lifting model developed by Wong and Booth (75,76), they found that the gastrocnemius muscle did not enlarge after 10 wk of 192 concentric contractions every third or fourth day. On the other hand, they found that the tibialis anterior (TA) muscle of these same animals enlarged 16-30% after the same training regimen. However, the TA muscle was performing eccentric instead of concentric contractions. It is therefore possible that muscle hypertrophy is a process of myofiber injury followed by repair or regeneration. The repeated process of injuring the muscle fibers through eccentric contractions followed by regeneration may result in an overcompensation of protein synthesis resulting in a net anabolic effect. This process would be akin to the overcompensation of muscle glycogen stores seen in muscle that is subjected to prolonged endurance training (44). Thus, the injury process may cause satellite cell proliferation resulting in the generation of new independent fibers, the repair of injured myofibers, and/or the fusion of myotubes with existing fibers causing hypertrophy and the maintenance of the nuclear-to-cytoplasmic ratio (74).

What role does injury have with regard to muscle fiber hyperplasia? The data is scant with regards to this question; however, studies by Winchester et al. (73) and Alway et al. (5) have shown that a temporal correlation exists between myofiber injury and muscle fiber hyperplasia during the first week of chronic stretch. During the first week of chronic stretch, there is a 27% increase in fiber number (5) concurrent with significant myofiber injury (i.e., 25-50% of all fibers exhibited morphological signs of injury) (74). After the first week of chronic stretch, the incidence of injury decreased dramatically despite the fact that fiber hyperplasia continued to occur (3,74). Giddings et al. (29) observed morphological signs of muscle fiber necrosis and regeneration such as pyknotic nuclei, myofibrillar disorganization, and sarcolemmal disruptions in resistance trained flexor carpi radialis muscle of the cat. This model of enlargement has been shown to result in fiber hyperplasia (35). However, these investigators (29) did not quantify the extent of injury. On the other hand, Antonio and Gonyea (8) showed that in the ALD muscle undergoing 28 d of progressive stretch overload, muscle fiber number increased 82% whereas the incidence of myofiber injury was small (i.e., 1-4% of the total fibers exhibited sarcolemmal disruptions). Nevertheless, it is apparent that under certain circumstances (e.g., chronic stretch) fiber hyperplasia is closely related to injury; however, other factors must explain the occurrence of fiber hyperplasia (i.e., tension, rapid growth, fiber splitting after a critical size is attained) when there is little myofiber injury.

Summary and future directions. It is evident that high-tension overload of adult skeletal muscle results in a significant muscle mass increase. This muscle mass increase is due primarily to an increase in fiber cross-sectional area and, to a lesser degree, fiber number (Tables 1, 2, and 3). Also, fiber length has been found to increase in avian muscle subjected to a stretch overload. Conversely, a decrease in contractile activity has been shown to result in a decrease in fiber cross-sectional area and number (57).

Numerous factors affect the adaptive response of muscle to overload and these factors differ widely among the numerous models used to study this phenomenon. The frequency, duration, and intensity (load or weight applied) of the stimulus has a profound impact on how muscle adapts. In light of this, it becomes clear why certain models of muscle hypertrophy result in a hypertrophic and/or hyperplastic response.
### TABLE 1. Quantitative evidence supporting muscle fiber hyperplasia—total fiber number determined histologically or after nitric acid digestion.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Overload Protocol/Treatment Duration</th>
<th>Subject</th>
<th>Muscle</th>
<th>Fiber Counting Technique</th>
<th>Muscle Mass</th>
<th>Mean Fiber Area</th>
<th>Fiber Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaughn and Goldspink (71)</td>
<td>Tenotomy/208 d</td>
<td>Mice</td>
<td>Soleus</td>
<td>Histologic cross-sections</td>
<td>46% increase</td>
<td>5% increase</td>
<td>10-33% increase</td>
</tr>
<tr>
<td>Sola et al. (64)</td>
<td>Chronic stretch/42-56 d</td>
<td>Chicken</td>
<td>ALD</td>
<td>Histologic cross-sections</td>
<td>120-180% increase</td>
<td>130% increase</td>
<td>16% increase</td>
</tr>
<tr>
<td>Alway et al. (4)</td>
<td>Chronic stretch/30 d</td>
<td>Quail</td>
<td>ALD</td>
<td>Nitric acid digestion and histologic cross-sections</td>
<td>172% increase</td>
<td>57% increase</td>
<td>52-74% increase</td>
</tr>
<tr>
<td>Alway (3)</td>
<td>Chronic stretch/30 d</td>
<td>Quail</td>
<td>ALD</td>
<td>Nitric acid digestion and histologic cross-sections</td>
<td>150% increase</td>
<td>50% increase</td>
<td>44% increase</td>
</tr>
<tr>
<td>Alway et al. (5)</td>
<td>Progressive intermittent/stretch 37 d</td>
<td>Quail</td>
<td>ALD</td>
<td>Nitric acid digestion and histologic cross-sections</td>
<td>64% increase</td>
<td>29% increase</td>
<td>27% increase</td>
</tr>
<tr>
<td>Antonio and Gonyea (8)</td>
<td>Weight lifting/298 d</td>
<td>Cat</td>
<td>FCR</td>
<td>Histologic cross-section</td>
<td>319% increase</td>
<td>35% increase</td>
<td>82% increase</td>
</tr>
<tr>
<td>Gonyea (33)</td>
<td>Weight lifting/707 d</td>
<td>Cat</td>
<td>FCR</td>
<td>Nitric acid digestion and histologic cross-section</td>
<td>16-44% increase</td>
<td>11% increase</td>
<td>20% increase</td>
</tr>
<tr>
<td>Ho et al. (38)</td>
<td>Weight lifting/55 d</td>
<td>Cat</td>
<td>FCR</td>
<td>Nitric acid digestion and histologic cross-section</td>
<td>11% increase</td>
<td>9% increase</td>
<td>12% increase</td>
</tr>
<tr>
<td>Tamaki et al. (66)</td>
<td>Left-right lower extremities (accidental death)</td>
<td>Rat</td>
<td>Plantaris</td>
<td>Nitric acid digestion</td>
<td>14% increase</td>
<td>0% increase</td>
<td>0% increase</td>
</tr>
<tr>
<td>SLVestrom et al. (62)</td>
<td>Weight lifting/84 d</td>
<td>Human</td>
<td>TA</td>
<td>Histologic cross-section</td>
<td>1.5% increase</td>
<td>14% increase</td>
<td>10% increase</td>
</tr>
</tbody>
</table>

ALD, anterior latissimus dorsi; FCR, flexor carpi radialis; TA, tibialis anterior; NM, not measured.

### TABLE 2. Qualitative or indirect evidence supporting muscle fiber hyperplasia.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Overload Protocol/Treatment Duration</th>
<th>Subject</th>
<th>Muscle</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yamada et al. (77)</td>
<td>Synergist ablation/7 d</td>
<td>Rat</td>
<td>Plantaris</td>
<td>Reported the presence of small fibers.</td>
</tr>
<tr>
<td>Chalmers et al. (18)</td>
<td>Synergist ablation/84 d</td>
<td>Cat</td>
<td>Plantaris</td>
<td>Reported the presence of small fibers.</td>
</tr>
<tr>
<td>Kennedy et al. (42)</td>
<td>Chronic stretch/10 d</td>
<td>Chicken</td>
<td>ALD</td>
<td>Reported the presence of small fibers located extrafascicularly that express an embryonic form of myosin heavy chain.</td>
</tr>
<tr>
<td>McCormick and Schuttz (51)</td>
<td>Chronic stretch/7 d</td>
<td>Chicken</td>
<td>ALD</td>
<td>Reported the presence of small fibers that expressed ventricular myosin heavy chain. These small fibers also had a high nuclear density and a high nuclear labeling index.</td>
</tr>
<tr>
<td>Mikesky et al. (53)</td>
<td>Weight lifting/1050 d</td>
<td>Cat</td>
<td>PLM</td>
<td>Found that the increase in muscle fiber area (11%) did not fully account for the total muscle mass increase of 24%. Because fiber length and fiber density remain the same, it is plausible that increases in fiber number contribute to the overall mass increase.</td>
</tr>
<tr>
<td>Giddings and Gonyea (30)</td>
<td>Weight lifting/581-2177 d</td>
<td>Cat</td>
<td>FCR, PLM</td>
<td>Reported the presence of small fibers.</td>
</tr>
<tr>
<td>Nygaard and Nielsen (56)</td>
<td>Swimming</td>
<td>Human</td>
<td>Deltoid</td>
<td>Cross-sectional study comparing swimmers with untrained controls. The swimmers had smaller Type I and IIa fiber areas than control despite greater muscle size.</td>
</tr>
<tr>
<td>Tesch and Larsson (67)</td>
<td>Bodybuilding</td>
<td>Human</td>
<td>Vastus lateralis</td>
<td>Cross-sectional study comparing the mean fiber area of the vastus lateralis m. and deltoide in bodybuilders and a control group. No differences in mean fiber area despite the greater lean body mass (i.e., muscle mass) and less body fat observed in the bodybuilders. An increased fiber number may explain the differences in muscle size. Genetic inheritance or training?</td>
</tr>
<tr>
<td>MacDougill et al. (47)</td>
<td>Bodybuilding/powerlifting</td>
<td>Human</td>
<td>Triceps brachii</td>
<td>Cross-sectional study comparing international caliber bodybuilders and powerlifters with a weight-matched group of swimmers. The bodybuilders and powerlifters had on average arm circumferences 27% greater than the controls yet the mean fiber area for both fast-twitch and slow-twitch muscle fibers were not different between the groups.</td>
</tr>
<tr>
<td>Larsson and Tesch (43)</td>
<td>Bodybuilding</td>
<td>Human</td>
<td>Vastus lateralis</td>
<td>Cross-sectional study comparing bodybuilders to age-matched controls. Bodybuilders’ possessed thigh circumference measurements which were 19% greater than controls yet mean fiber cross-sectional area was not different.</td>
</tr>
<tr>
<td>Alway et al. (5)</td>
<td>Bodybuilding</td>
<td>Human</td>
<td>Biceps brachii</td>
<td>Cross-sectional study comparing elite male and female bodybuilders. Biopsies cross-sectional area was positively correlated with fiber cross-sectional area (R = 0.75) and fiber number (R = 0.55).</td>
</tr>
</tbody>
</table>

ALD, anterior latissimus dorsi; PLM, palmaris longus muscle; FCR, flexor carpi radialis.
TABLE 3. Studies showing no change in fiber number—total fiber number determined histologically or after nitric acid digestion.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Overload Protocol/Treatment Duration</th>
<th>Subject</th>
<th>Muscle</th>
<th>Fiber Counting Technique</th>
<th>Muscle Mass</th>
<th>Mean Fiber Area</th>
<th>Fiber Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Golink et al. (31)</td>
<td>Synergist ablation/exercise-28-280 d</td>
<td>Rat</td>
<td>Soleus plantaris, EDL</td>
<td>Nitric acid digestion</td>
<td>25-88% increase</td>
<td>NM</td>
<td>NC</td>
</tr>
<tr>
<td>Timson et al. (70)</td>
<td>Synergist ablation/42-56 d</td>
<td>Mice</td>
<td>Soleus</td>
<td>Nitric acid digestion</td>
<td>31-47% increase</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Golink et al. (32)</td>
<td>Chronic stretch/6-65 d</td>
<td>Chicken</td>
<td>ALD</td>
<td>Nitric acid digestion</td>
<td>22-25% increase</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Antonio and Golaya (7)</td>
<td>Intermittent stretch/15 d</td>
<td>Qual</td>
<td>ALD</td>
<td>Histological cross-section</td>
<td>52% increase</td>
<td>28% increase</td>
<td>NC</td>
</tr>
<tr>
<td>Yarasheki et al. (78)</td>
<td>Weight lifting/56 d</td>
<td>Rat</td>
<td>Rectus femoris</td>
<td>Histological cross-section</td>
<td>8% increase</td>
<td>0-20% increase</td>
<td>NC</td>
</tr>
</tbody>
</table>

Ten days of surgically induced stretch produced a 29% muscle mass increase. Frequency histogram of fiber cross-sectional area shows that the muscle mass increase was due to fiber hypertrophy. Changes in muscle mass could be accounted for by changes in muscle length and fiber cross-sectional area. Same method of stress as Barnett et al. (13). Cross-sectional study comparing powerlifters, distance runners, and untrained subjects showed that the powerlifters had significantly larger mean fiber areas than the other two groups. Fiber number was not estimated, albeit the large differences in fiber area may account for the differences in muscular development.

For example, the magnitude of the muscle mass increase in the ALD muscle of adult quail far exceeds those reported for exercise and compensatory hypertrophy models. This is meaningful in that fiber hyperplasia is a consistent and significant feature of stretch overloaded muscle. In the avian stretch model, it is easy to control the duration, frequency, and the load or weight applied to the muscle; hence, the degree of overload is not dependent on the volition of the animal.

It should be noted though that the avian stretch model presents a stimulus that differs greatly from exercise (i.e., resistance training). Further work is needed to resolve the issue as to whether or not a model of muscle enlargement has to mimic a physiologic model (i.e., exercise). Are the basic mechanisms of muscle hypertrophy similar between species such that relevant information can be obtained from nonphysiologic models (e.g., stretch, synergist ablation)? Is the adaptive response of stretched ALD muscle merely a reflection of the chronic and relentless nature of the stimulus? Would human muscle respond in a similar manner if subjected to such a stimulus? Certainly, the latter question is not likely to be answered since it would be unethical to administer such a treatment to human subjects. If the basic mechanisms governing skeletal muscle hypertrophy and hyperplasia are similar between species regardless of the stimulus paradigm, then the avian stretch model would be a suitable model.

Using this model, it is possible to uncouple the processes of fiber hypertrophy and hyperplasia and therefore study the distinct cellular processes that contribute to each (8).

Alternatively, the ideal model for studying the role of fiber hyperplasia in exercise-induced muscle enlargement should have the following qualities: 1) the training protocol should mimic the human condition (i.e., brief, intermittent, high force muscle contractions interspersed with a prolonged rest interval); 2) the ability to train a muscle of one limb while the muscles of the other limb serve as an intra-animal control thus enabling precise comparisons of fiber number between the exercised and control limb muscles; 3) the training program needs to be long-term in nature since human bodybuilders often reach their extreme muscular development only after several years of intense training; and 4) gains in muscle mass are consistent and predictable.

The model that most closely meets the aforementioned criteria for an studying muscle enlargement is the cat weight lifting model (34). This model has demonstrated modest increases in fiber number (9%) (35); however, due to its lack of predictability (i.e., some of the cat's muscles hypertrophied tremendously (100%) whereas others showed no increase in muscle mass), it still has serious limitations as a model to study hypertrophic mechanisms. If the training variables that are the best predictors of muscle hypertrophy are controlled
precisely (i.e., speed of movement and load lifted) (52), then this model would be ideal for studying the mechanisms of exercised-induced muscle hypertrophy.

Unlike the avian stretch model, exercise and compensatory hypertrophy models depend on the volition of the animal. In animal resistance training models, it is more difficult to control for the speed of movement and the load imposed on the muscle. In the compensatory hypertrophy model, the amount of overload is dependent on the decision of the animal to weight bear. These discrepancies provide a reasonable explanation for the dissimilar results found in experiments from different laboratories. Thus, the occurrence of fiber hyperplasia may simply be a reflection of the stimulus magnitude and duration.

Useful information concerning the role of fiber hypertrophy and hyperplasia in adult skeletal muscle enlargement has been acquired from the different animal models as well as from cross-sectional studies of human athletes. The current scientific evidence suggests that muscle fiber hyperplasia is a consistent feature of certain perturbations (i.e., stretch) whereas other methods of overload (i.e., compensatory hypertrophy) generally produce no changes in fiber number. Certainly, if one is interested in studying the mechanisms of new fiber formation, the compensatory hypertrophy model would not be suitable. Exercise-induced muscle hypertrophy also produces mixed results in terms of the relative contribution of fiber hyperplasia. However, until the training variables that best predict gains in fiber area and number are delineated, exercise models will have strict limitations with regard to studying muscle growth. Nevertheless, adult skeletal muscle has the capacity to increase fiber number. The mechanisms for fiber hyperplasia are likely the result of two processes: satellite cell proliferation and longitudinal fiber splitting (5, 9, 73, 74).

The illustration for Figure 1 was done by Talmid Brown, Medical Illustration, Southwestern Medical Center, Dallas, TX.

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