Regrowth of Skeletal Muscle Atrophied from Inactivity

SHUICHI MACHIDA[1] and FRANK W. BOOTH[1–3]

[1]Departments of Biomedical Sciences and of [2]Medical Pharmacology and Physiology, and [3]Dalton Cardiovascular Institute, University of Missouri, Columbia, MO

ABSTRACT

MACHIDA, S., and F. W. BOOTH. Regrowth of Skeletal Muscle Atrophied from Inactivity. *Med. Sci. Sports Exerc.*, Vol. 36, No. 1, pp. 52–59, 2004. The current state of knowledge regarding regrowth of skeletal muscle after inactivity-induced atrophy is reviewed. Muscle regrowth is incomplete after hindlimb suspension in juvenile rats and after limb immobilization in old animals. The process of regrowth from immobilization-induced atrophy likely involves the reversal of directional changes in molecules producing muscle loss while initiating anabolic processes for regrowth of muscle mass. Unfortunately, the molecular mechanisms responsible for successful, or failed, muscle regrowth are not well understood. The purpose of the review is to provide current knowledge about the biology of muscle regrowth from inactivity-induced atrophy. **Key Words:** AGING, ATROPHY, GROWTH, HYPERTROPHY, IMMOBILIZATION, REHABILITATION

The contribution of skeletal muscle strength and mass to health is under-recognized, where losses result in an increased incidence of death (45). For example, in men ≥60 yr, lower grip strength values were associated with an increased risk of mortality (45). Muscle mass is lost through physical inactivity. A sedentary lifestyle culminates in premature physical frailty (64). Furthermore, immobilization of limbs produces a rapid loss of muscle mass (13). Molecular links between mechanical unloading/reloading and muscle wasting/regrowth from these inactivity-type conditions are needed for patient care. The speculation is made that such medical evidence would encourage individuals, including patients, to undertake more preventive care, i.e., work either to regain muscle strength after rapid losses of muscle mass, such as in limb immobilization, or work to prevent loss in muscle mass for more prolonged periods (such as sedentary living in nursing homes).

Muscle regrowth after hindlimb unloading and limb immobilization is not complete in animals at certain stages of life (18,51,73). Remarkably, muscles of old animals exhibit little regrowth from atrophy after limb immobilization (18,73). Unfortunately, the molecular mechanisms responsible for successful, partial, and failed muscle regrowth from inactivity-induced atrophy are not well understood. The purpose of this review is to describe the current knowledge regarding regrowth of skeletal muscle atrophied from physical inactivity. To understand which inactivity-induced atrophy processes require reversal to allow muscle regrowth, a brief summary of changes that occur with inactivity-induced atrophy will be reviewed first. [Muscle atrophy in spinal cord injury has been reviewed elsewhere (23) and will not be covered here.]

INACTIVITY INDUCES MUSCLE ATROPHY

Skeletal muscle undergoes rapid and profound atrophy in response to decreased mechanical loading, e.g., in limb immobilization (13), hindlimb suspension (48,67), bed rest (9), and spaceflight (5,8,26,31) (For the purposes of this review, the generic term "inactivity-induced atrophy" will be used to differentiate these conditions from muscle wasting produced by fasting, glucocorticoids, etc.). It is also contended that inactivity-induced atrophy and growth inhibition do not have identical mechanisms. For the purposes of this review, atrophy is defined as a decrease in muscle mass from initial mass. As such, prevention of maturational growth is not atrophy. For example, the interruption of growth in juvenile rats occurs mainly through an interruption in myonuclear accretion (22,61) when compared with that in aged-matched control rats, whereas in mature or old rats, muscle atrophy occurs mainly through a loss in existing mass, including a reduction in DNA unit size (cytoplasm-to-myonuclei ratios) (7,39) and a loss of myonuclei (7). In addition, inactivity-induced atrophy suppresses satellite cell proliferation in vivo (22,51,61) and *in vitro* (18).

Comparisons of the models of hindlimb suspension and immobilization. Limb immobilization is often used in orthopedic medicine. For example, after total hip arthroplasty and rehabilitation, muscle wasting persisted 5 months postoperatively in 64-yr-old patients (53). Hindlimb suspension is employed to mimic spaceflight (67). Although

Address for correspondence: Frank W. Booth, Ph.D., Department of Biomedical Sciences, E102 Vet. Med. Bldg., University of Missouri, 1600 E. Rollins, Columbia MO 65211; E-mail: boothf@missouri.edu.
Submitted for publication March 2003.
Accepted for publication August 2003.

0195-9131/04/3601-0052
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/01.MSS.0000106175.24978.84

both models produce smaller skeletal muscles as compared to untreated animals, the degree of inactivity differs. Both models unload skeletal muscles, i.e., muscles are nonweightbearing, but unloaded isotonic contractions are allowed only in the suspension model. As a consequence, EMG from hindlimb muscles is only transiently suppressed during suspension, returning toward control values by the 7th day of suspension (4). In contrast, the EMG from immobilized muscles fixed in their shortened position remains reduced by 85–90% of control (25,32). Although both models produce up to 50% atrophy in predominantly slow muscles, only in the limb immobilization, when muscles are fixed at resting or shorter than resting lengths, does the percentage of atrophy in fast-type muscles approach that of slow muscle (12). In contrast, ~15–20% of fast muscle weight is lost in suspended limbs (67). Because of these differences, the model of suspension is preferred as an Earth-based model to mimic skeletal muscle function during spaceflight with the rationale that muscles of astronauts undergo unloaded, isotonic contractions (48). However, the loss in fiber area is 2–3 times greater in Type I than Type II fibers in the tail suspension model. In contrast, the mean fiber cross-sectional areas were 16–36% smaller after the 11-d flight with the relative effect being type IIB > IIA > I (24), which is opposite hindlimb suspension. Thus, there should be no preferred model of muscle atrophy. The authors disagree with review groups which dictate exclusivity to which atrophy model to use. For example, NASA has emphasized research conformance to the hindlimb suspension model even with the discrepancy between human spaceflight and ground-based data for percentage cross-sectional area for Type II fibers. We believe such research mandates short-circuit creativity and limit insights as more information can be obtained from a comparison of multiple models than from all investigators applying the same model.

Protein synthesis decreases in inactivity-induced muscle atrophy. Inactivity-induced atrophy, such as hindlimb unloading and limb immobilization, results in the loss of muscle mass. Five days of hindlimb unloading resulted in decreases in total RNA and protein synthesis in soleus and gastrocnemius muscles (28). Some studies infer that both transcriptional and translational rates are decreased in inactivity-induced atrophying muscle, depending upon the time of measurement (67). The rates of mixed protein synthesis in rat gastrocnemius muscle declined in the first 6 h of limb immobilization (15). Watson et al. (70) and Morrison et al. (50) showed that the rate of α-actin and cytochrome c protein synthesis decreased whereas their mRNA contents were unchanged in rat gastrocnemius muscle in the first 6 h of hindlimb immobilization, implying a translational mechanism was regulating protein content. In addition, Morrison et al. (50) also reported decreases in the nanograms of cytochrome c protein synthesized per day, cytochrome c mRNA, and in cytochrome c protein of the red quadriceps muscle after 7 d of immobilization, implying regulation may be occurring pretranslationally. Thus, the loss of cytochrome c and actin proteins early in inactivity-induced muscle atrophy may occur by a translational change, whereas their loss later in muscle atrophy may be, in part, due to a decrease in pretranslational mechanisms.

Protein degradation increases in inactivity-induced muscle atrophy. From the percentage changes in protein synthesis rates and in muscle mass, protein degradation rates were deduced to increase in unloaded muscles (28,66), and some factors potentially contributing to these increases will be briefly mentioned next. The protein xanthine oxidase, which produces superoxides is increased in muscle cytoplasm of immobilized limbs, enhancing oxidative stress (42). The resultant oxidative stress increases tumor necrosis factor-α (TNF-α), which promotes loss of muscle protein (54). TNF-α triggers the activation of transcription factor nuclear factor-κB (NF-κB), which could enhance ubiquitin-dependent proteolysis in muscles (44). NF-κB family member p50, but not p65, was activated in unloaded soleus muscle (33). These data have suggested that inactivity-induced oxidative stress would induce increases in protein degradation via TNF-α/NF-κB/ubiquitin-dependent proteolytic system.

For most proteins, degradation by the proteasome requires ubiquitination by a specific ubiquitin-protein ligase (E3) (36). Atrogin, an E3 ubiquitin ligase, was increased in rat medial gastrocnemius muscle undergoing hindlimb unloading (29) or immobilization (unpublished data). Mice deficient in atrogin were found to be resistant to muscle atrophy (10). Jagoe et al. (36) concluded that atrogin-1 appears to be uniquely regulated or at least to be one of only a small set of ubiquitin-protein ligases that play a key role in the accelerated proteolysis in atrophying muscles.

Myonuclear number decreases in inactivity-induced muscle atrophy. Myonuclear number decreases in inactivity-induced atrophying muscle (5,31,47), coincident with the decrease in muscle fiber size. However, a fiber type difference in myonuclear loss exists. A greater decrease in myonuclear number in Type I (soleus) than in Type II (plantaris) myosin heavy chain (MHC)-expressing fibers occurred in response to inactivity [hindlimb suspension or spaceflight (5,7,31,47)]. An increase in the nuclear apoptotic marker, terminal deoxynucleotidyl transferase histochemical staining, in unloaded (6) muscles suggests that apoptosis contributes to the elimination of myonuclei and/or satellite cells from atrophying fibers. However, the molecular mechanisms by which inactivity induces apoptosis in skeletal muscle are not well understood. An unanswered question is whether apoptosis causes inactivity-induced atrophy of muscle or is a result of muscle atrophy. Myocyte apoptosis triggered by high levels of circulating TNF-α has been described, suggesting that TNF-α could be involved in triggering cell death (20).

REGROWTH OF SKELETAL MUSCLE ATROPHIED FROM INACTIVITY-INDUCED ATROPHY

Mitchell and Pavlath (47) suggested that mechanisms of regrowth may differ from postnatal growth and hypertrophy in the adult, in part, due to the need to reverse the mechanisms that caused a reduction in muscle mass. This section

will consider mechanisms by which muscle regrows from an inactivity-induced atrophy.

Protein synthesis increases in regrowing muscle. Rates of mixed protein synthesis in rat gastrocnemius muscle significantly increased to control values during the first 6 h after a 7-d period of limb immobilization, then remained at control values for the next 2 d, and finally significantly increased to about twice the control values on the 4th day after immobilization (68). Cytochrome c protein synthesis and mRNA levels were decreased by 19% and 40%, respectively, after 7 d of hindlimb immobilization (50). After ending hindlimb immobilization, the rate of cytochrome c synthesis and its mRNA did not exceed control values during the first 2 d of recovery from immobilization. However by the 4th day of recovery, the rate of cytochrome c protein synthesis was 92% higher than control, and the amount of cytochrome c mRNA had returned to control values. A similar pattern to the cytochrome c recovery was followed by actin protein synthesis rates, which were 33%, 40%, 100%, and 300% of a percentage of control values at 0 h, 6 h, 2 d, and 4 d of recovery after 7 d of hindlimb immobilization (49). α-actin mRNA levels were reduced 42% at the 0-recovery time point and returned to control values by the 2nd day of recovery and were maintained at control values on the 4th recovery day (49). The greater percentage increase in protein synthesis rates than increases in mRNA and protein levels for cytochrome c and α-actin implies that recovery from atrophy is a process that includes pretranslational, translational, and posttranslational regulations. Similar conclusions exist for resistance training when rat muscles contract against external loads (71,72).

Protein degradation increases in regrowing muscle. Indirect estimates (from protein synthesis and concentration values) for protein degradation rates remain elevated during initial phases of recovery from prior inactivity-induced atrophy. Enhanced oxidative stress occurred during the 5-d of recovery from immobilization-induced muscle atrophy (41). Large numbers of lysosomal-like bodies were observed in old skeletal muscles during their 4-wk recovery from 4 wk of immobilization-induced muscle atrophy (73). Increases in cytochrome c and actin protein synthesis rates greatly exceeded the rate of gain in the contents of these proteins (49,50), implying an increase in their degradation rates.

Muscle precursor cell proliferation increases in regrowing muscle. Muscle precursor cells are necessary for postnatal development, hypertrophy, and regeneration of skeletal muscle. Recently, it has become clear that subpopulations of satellite cells can differentiate to cell types other than muscle, and thus the term "muscle precursor cell" has been used for those satellite cells that can become myoblasts. In this review, the terminology of satellite cell or muscle precursor cell is employed as used in the cited reference. Early postnatal muscle growth is associated with an increase in myonuclear number, and additional myonuclei are provided by myoblast proliferation and fusion to the fibers during postnatal development. Adult animals undergoing functional overload also require a satellite cell-induced increase in myonuclear number as a necessary component of full hypertrophic growth in adult muscle (2,56). Schiaffino et al. (59,60) demonstrated that satellite cell proliferative activity is increased during the early stages (3 d) of the compensatory hypertrophy of the rat soleus or extensor digitorum muscle that accompanies synergist ablation. These observations lead to the question of whether the proliferation, differentiation, and fusion of satellite cells with existing muscle fibers are required for recovery of muscle mass after atrophy produced by reduced mechanical loading.

Myonuclear number increases in regrowing muscle. During recovery of soleus muscle mass upon its reloading, the 35% decrease in myonuclear number after 2 wk of hindlimb unloading was restored to control values (47), suggesting that myogenic precursor cells can proliferate and fuse with myofibers during the recovery process consisting of enlargement of atrophied fibers. Myonuclear number was defined as the number of myonuclei per 100 myofibers (47). However, inhibition of muscle precursor cell proliferation by local γ-irradiation completely prevented the recovery in soleus muscle mass and myonuclear number (47). The first week of soleus myofiber regrowth after hindlimb unloading is mediated by myofiber-intrinsic processes, independent of the calcineurin signaling pathway (46). During the second week of soleus muscle regrowth, myonuclear accretion that is calcineurin-dependent was necessary for regrowth. Regrowth of plantaris myofibers occurred without myonuclear accretion as no myonuclei were lost in these unloaded muscles, suggesting that exclusively myofiber-dependent processes contributed to regrowth of the plantaris after atrophy (46). The lack of a necessity for satellite cell addition to the recovering plantaris muscle may be due to the lesser atrophy of the plantaris than the soleus muscle, thereby eliminating the requirement for muscle precursor cell during the recovery process. However, calcineurin activity was required for growth during both of the first 2 wk of plantaris muscle regrowth from atrophy (46).

Satellite cell mitotic activity has been shown to be a prerequisite for myofiber enlargement in growth (52) and hypertrophy (56). The mitotic activity of satellite cells was higher during the 2 wk immediately after the resumption of weight bearing in the soleus muscles from hindlimb-suspended rats compared with soleus muscles from the control rats (51). It is likely that the increased functional demands placed on the reloaded muscle stimulated satellite cell proliferation and subsequently myonuclear accretion (21). Potential sources of factors stimulating regrowth of atrophied muscle fibers are growth factors induced by either mechanochemical transduction or myofiber damage (35,43,65). Reloading induces mechanical forces that become transmitted though the extracellular matrix, integrins, and cytoskeleton, potentially

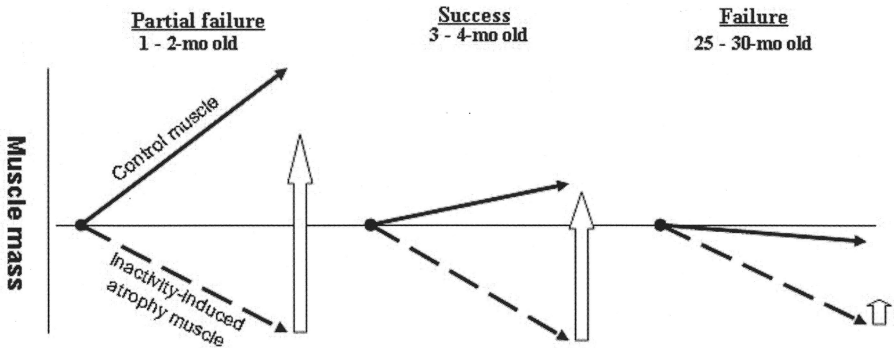

FIGURE 1—Age-related differences in the regrowth of skeletal muscle atrophy from inactivity. The *solid line* indicates change in muscle mass of controls during the experimental period. The *broken line* indicates the loss of muscle mass during inactivity-induced atrophy. The height of the *vertical open arrow* approximates regrown muscle mass in recovery from atrophy. (A) Before regrowth from inactivity, skeletal muscles of juvenile rats had smaller myofiber diameters and a smaller muscle-weight to body-weight ratios compared with the muscles at the beginning of the inactivity period, indicating that the inactivity not only stopped skeletal muscle growth but also induced an absolute atrophy in the juvenile skeletal muscles (51). The majority of the difference at the end of atrophy in juvenile muscles is due to prevention of growth. After the end of inactivity, juvenile skeletal muscles exhibit unsuccessful catch-up growth compared with the age-matched control animals. Most of the atrophy in younger adult muscles is true loss from preatrophy weight and regrowth is nearly complete. In contrast, control old skeletal muscle is sarcopenic (atrophying) and has minimal or no regrowth from true loss compared to preatrophy values. It is possible that satellite cells in old atrophy muscle are unable to contribute to the regrowth during recovery period from inactivity due to a lack of growth factor stimulation (18).

signaling ribosomes to activate translation and/or transmit forces to the nuclear scaffolds, possibly altering transcriptional regulation (35). Markers of myofiber damage, such as the infiltration of specific subpopulations of macrophages into necrotic fibers (43,65), sarcolemmal disruptions (38), and myofibrillar lesions (69), have been reported shortly after the resumption of weight bearing from hindlimb unloading. An increased incidence of macrophage-invaded muscle fibers has also been reported at 48 h of reloading after a 10-d spaceflight (55). Macrophages secrete substances, such as platelet-derived growth factor, basic fibroblast growth factor, leukemia-inhibitory factor, and insulin-like growth factor (IGF-I), all known to stimulate muscle-precursor-cell mitotic activity (30,63). It is possible that low-level myofiber injury may stimulate satellite cell proliferation after the resumption of weight bearing to aid the compensatory regrowth response of the muscle.

IMPAIRED REGROWTH AFTER ENDING DECREASED MECHANICAL LOADING

Aging. Gerontological studies classify the stages of life in Fischer 344/Brown Norway F1 generation cross male rats as: juvenile/young (1–2 months old), young (2–4 months old), adult (18 months old), and old (>30 months old).

Juvenile skeletal muscle. Skeletal muscles from 1- to 2-month-old rats have unsuccessful catch-up growth after an inactivity-induced atrophy compared with the age-matched control rats (panel labeled as "partial failure" in Fig. 1). The soleus muscle of 1- to 2-month-old rats failed to achieve the muscle size of their age-matched, nontreated controls after 9 wk of reloading after a 4-wk period of hindlimb suspension, even though body weights had reached the same size as age-matched, weight-bearing, untreated rats (51). Before regrowth from the hindlimb suspension, soleus muscles had smaller myofiber diameters and a smaller muscle-weight to body-weight ratios compared with soleus muscles from rats at the beginning of suspension, indicating that hindlimb suspension not only inhibited skeletal muscle growth during immobilization but also resulted in an absolute atrophy in the juvenile soleus muscle, i.e., reduced from initial mass (51). Catch-up to an equivalent number of satellite cells and myonuclei, and equivalent fiber volume in age-matched controls also failed to occur in the soleus muscle of 1- to 2-month-old rats who had undergone a 10-d period of hindlimb suspension (22). These reports imply that an inhibition of satellite cell function by inactivity-induced atrophy may limit regrowth of muscle mass and myonuclear number in 1- to 2-month-old rats as postnatal muscle growth is associated with an increase in myonuclear number from satellite cells (22,40).

Young skeletal muscle. In 3- to 4-month-old rats, when the rapid phase of growth has slowed, successful regrowth from immobilization-induced atrophy was exhibited (14; labeled as "success" in Fig. 1).

Old skeletal muscle. However, muscles of 25- to 30-month-old rats did not regrow after atrophy by limb immobilization (18,73; labeled as "failure" in Fig. 1). A comparison of young (14) and old (18,73) rats suggests that the mechanisms of regrowth in old atrophied muscle may differ from that in young atrophied muscle. Satellite cell number and proliferative potential decreases with age (62). Inactivity suppresses satellite cell mitotic activity in skeletal muscle (22,51,61) and also decreases myonuclear number in adult rats (5,7,31). The above observations generated a hypothesis that satellite cells in old atrophied muscle might not be in sufficient quantity to contribute to the regrowth during recovery period from inactivity (18). However, Chakravarthy et al. (18) showed that direct IGF-I administration onto an atrophied muscle promoted both *in vitro* satellite cell proliferation

and *in vivo* regrowth of skeletal muscle from limb immobilization in 26- to 30-month-old rats. Many growth factors can enhance satellite cell proliferation (30). The above data prompted a new hypothesis that satellite cells in skeletal muscle of 26- to 30-month-old rats were in sufficient quantity, but quiescent, or inactive due to lack of a some unknown "growth factor milieu" or that satellite cells were less responsive to endogenous levels of growth factors (Fig. 2). Two lines of reasoning suggest that growth factors could be limiting to regrowth of old skeletal muscle. First, the rescue by IGF-I of regrowth and *in vitro* satellite cell proliferation in old muscle that was atrophied by inactivity (18) suggests endogenous growth factors may not be in abundance in the old muscle. Second, old skeletal muscle regenerates completely after its destruction with myotoxins (16). Muscle regeneration is associated with an upregulation of growth factors and a successful return of muscle to its premyotoxin size (34,37), whereas undamaged atrophy muscle in immobilized limbs does not regrow, possibly because of the lack growth factor upregulation as seen in muscle regenerating from myotoxin damage.

THE CELLULAR AND MOLECULAR MECHANISMS IN REGROWING MUSCLE

The cellular and molecular mechanisms that contribute to the regrowth of muscle mass after reduced mechanical loading are just beginning to be delineated. As most studies of muscle regrowth from atrophy produced by limb immobilization have been performed in rats, the vast amount of literature on mouse satellite cells in the regenerating muscles after toxin damage will not be reviewed here as regeneration is beyond the review's scope. However, no studies within the same lab comparing rat and mouse satellite cells during recovery from inactivity-produced muscle atrophy are known.

It is likely that satellite cells play essential role for successful regrowth from inactivity-induced muscle atrophy. Because skeletal muscle fibers are terminally differentiated (postmitotic) and require the proliferation of satellite cells to provide new myonuclei for increased muscle regrowth (46), factors stimulating satellite cells are likely produced locally in regrowth. IGF-I and leukemia inhibitory factor (LIF) are local factors, which are expressed during muscle growth (3,58) and can induce proliferation of satellite cells (18,63). However, the signaling mechanisms by which these factors activate satellite cells are just beginning to be understood. Chakravarthy et al. (17) found that IGF-I-induced enhancement of satellite cell proliferation is mediated via activation of PI3K/Akt pathway through down-regulation of a cell-cycle inhibitor $p27^{Kip\ 1}$. Bodine et al. (11) demonstrated that Akt/mTOR pathway, and its downstream target, $p70^{S6K}$, is implicated in muscle regrowth after atrophy. Childs et al. (19) have recently found that $p70^{S6K}$ phosphorylation was transiently increased during the 3rd recovery day after a 10-d period of limb immobilization (Fig. 3). During muscle regrowth induced by reloading, muscle damage can occur (43,65), inducing an inflammatory response within skeletal muscle. Spangenburg and Booth (63) showed that LIF induces *in vitro* satellite cell proliferation by activation of the JAK2-STAT3 signaling pathway. STAT3 phosphorylation was also increased in the soleus muscle during the 3rd and 6th recovery days after a 10-d period of limb immobilization (19). These results suggest that IGF-I and LIF might activate satellite cells during muscle regrowth induced by reloading after inactivity-induced atrophy.

Another potential stimulus for muscle regrowth is Ca^{2+}-signaling (Fig. 3). Calcineurin is a Ca^{2+}/calmodulin-dependent serine/threonine phosphatase that is activated in response to sustained increases in intracellular Ca^{2+} and is inhibited by cyclosporin A (CsA). Mitchell et al. (46) showed that CsA not only completely prevented regrowth of the atrophied plantaris muscle after hindlimb unloading but also led to further atrophy. On the other hand, CsA only attenuated regrowth of the soleus. Initial regrowth of the soleus muscle was by a calcineurin-independent pathway,

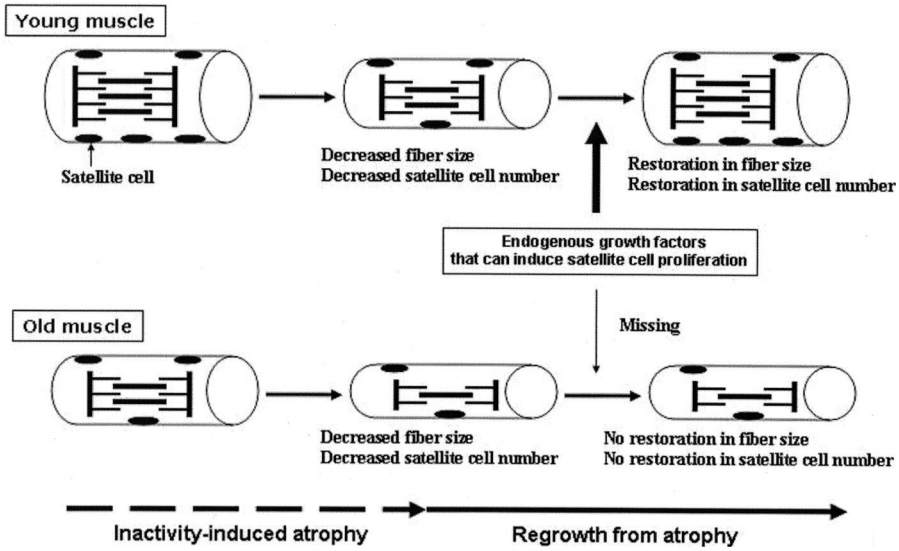

FIGURE 2—Proposed model for roles of endogenous growth factors in regrowing muscle after atrophy. Inactivity leads to muscle atrophy. Accompanied with this atrophy in both young and old soleus muscles is the loss of myonuclei and decrease in muscle fiber size. During regrowth of young soleus muscle after atrophy, growth factors likely upregulate, deduced from the age-matched control muscles being in a growth phase, producing satellite cell proliferation and fusion with myofibers allowing the enlargement of atrophied fibers. In contrast, no restoration in fiber size occurs during regrowth of the old muscle after an inactivity-induced atrophy. Our current hypothesis is that some ingredients of a growth factor milieu are missing to stimulate muscle growth in old skeletal muscle after inactivity-induced atrophy.

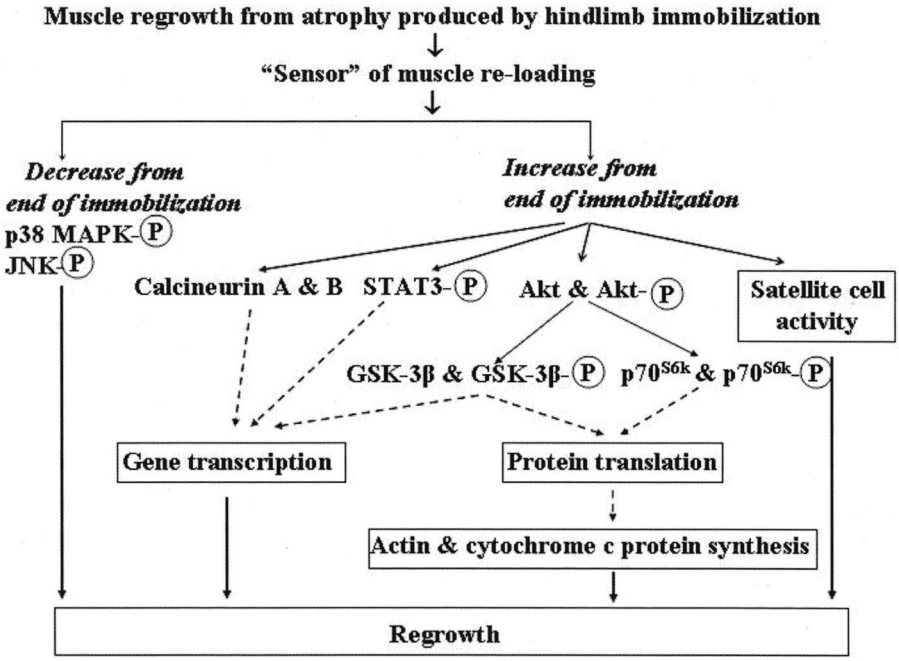

FIGURE 3—Schematic model of some signaling pathways activated in the atrophied soleus muscle after ending of limb immobilization in young rats. Data taken from refs. (14,18,19,46,49,50,68).

whereas in the 2nd week of recovery, regrowth and myonuclear accretion were calcineurin-dependent (46). Interestingly, calcineurin has been shown to be required for myoblast differentiation *in vitro* (1,27), allowing the speculation that CsA prevented either the activation of proliferated satellite cells or their fusion with preexisting fibers resulting in muscle regrowth. Calcineurin protein has been observed in BrdU-labeled, but not in quiescent, satellite cells of the bupivacaine-injected rat tibialis anterior muscle (57). Therefore, calcineurin has emerged as a potential key signaling molecule in skeletal regrowth.

SUMMARY

This review compiled the current knowledge regarding regrowth of skeletal muscle atrophied from inactivity. Mechanisms of skeletal muscle regrowth, such as the contribution of satellite cells and calcineurin, differ between fast and slow muscles. In addition, muscle regrowth mechanisms likely differ among muscles from juvenile, adult, and old subjects as the inhibition of growth and atrophy mechanisms must be reversed in juvenile muscles recovering from inactivity-induced atrophy. Regrowth from inactivity-induced atrophy in juvenile rats is a partial success, whereas regrowth is partially or fully incomplete in old muscle. In old skeletal muscles, both sarcopenic and inactivity-associated atrophy mechanisms must be reversed. Although low satellite cell numbers may contribute to the failure of old atrophied muscles to regrow from inactivity, the rescue with IGF-I of *in vitro* satellite cell proliferation and of *in vivo* muscle mass implies that growth factors may also be limiting in old muscle regrowth. Future studies need to determine molecular links for medical evidence to promote and improve the prescription of rehabilitation from muscle atrophy.

This review was written while supported by NIH AR19393 and AG 18780 (FB). Thanks to Espen Spangenburg, Ph.D., J. Scott Pattison, and Simon Lees, Ph.D., for critical reviews.

REFERENCES

1. ABBOTT, K. L., B. B. FRIDAY, D. THALOOR, T. J. MURPHY, and G. K. PAVLATH. Activation and cellular localization of the cyclosporine A-sensitive transcription factor NF-AT in skeletal muscle cells. *Mol. Biol. Cell.* 9:2905–2916, 1998.
2. ADAMS, G. R., V. J. CAIOZZO, F. HADDAD, and K. M. BALDWIN. Cellular and molecular responses to increased skeletal muscle loading after irradiation. *Am. J. Physiol. Cell Physiol.* 283:C1182–C1195, 2002.
3. ADAMS, G. R., F. HADDAD, and K. M. BALDWIN. Time course of changes in markers of myogenesis in overloaded rat skeletal muscles. *J. Appl. Physiol.* 87:1705–1712, 1999.
4. ALFORD E. K., R. R. ROY, J. A. HODGSON, and V. R. EDGERTON. Electromyography of rat soleus, medial gastrocnemius, and tibialis anterior during hind limb suspension. *Exp. Neurol.* 96:635–649, 1987.
5. ALLEN, D. L., W. YASUI, T. TANAKA, et al. Myonuclear number and myosin heavy chain expression in rat soleus single muscle fibers after spaceflight. *J. Appl. Physiol.* 81:145–151, 1996.
6. ALLEN, D. L., J. K. LINDERMAN, R. R. ROY, et al. Apoptosis: a mechanism contributing to remodeling of skeletal muscle in response to hindlimb unweighting. *Am. J. Physiol.* 273:C579–C587, 1997.
7. ALLEN, D. L., J. K. LINDERMAN, R. R. ROY, R. E. GRINDELAND, V. MUKKU, and V. R. EDGERTON. Growth hormone/IGF-I and/or resistive exercise maintains myonuclear number in hindlimb unweighted muscles. *J. Appl. Physiol.* 83:1857–1861, 1997.
8. BALDWIN, K. M. Effect of spaceflight on the functional, biochemical, and metabolic properties of skeletal muscle. *Med. Sci. Sports Exerc.* 28:983–987, 1996.

9. BERG, H. E., L. LARSSON, and P. A. TESCH. Lower limb skeletal muscle function after 6 wk of bed rest. *J. Appl. Physiol.* 82:182–188, 1997.
10. BODINE, S. C., E. LATRES, S. BAUMHUETER, et al. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* 294:1704–1708, 2001.
11. BODINE, S. C., T. N. STITT, M. GONZALEZ, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Natl. Cell. Biol.* 3:1014–1019, 2001.
12. BOOTH, F. W. Regrowth of atrophied skeletal muscle in adult rats after ending immobilization. *J. Appl. Physiol.* 44:225–230, 1978.
13. BOOTH, F. W. Effect of limb immobilization on skeletal muscle. *J. Appl. Physiol.* 52:1113–1118, 1982.
14. BOOTH, F. W., and M. J. SEIDER. Recovery of skeletal muscle after 3 mo of hindlimb immobilization in rats. *J. Appl. Physiol.* 47: 435–439, 1979.
15. BOOTH, F. W., and M. J. SEIDER. Early change in skeletal muscle protein synthesis after limb immobilization of rats. *J. Appl. Physiol.* 47:974–977, 1979.
16. CARLSON, B. M., and J. A. FAULKNER. The regeneration of noninnervated muscle grafts and marcaine-treated muscles in young and old rats. *J. Gerontol. A Biol. Sci. Med. Sci.* 51:B43–B49, 1996.
17. CHAKRAVARTHY, M. V., T. W. ABRAHA, R. J. SCHWARTZ, M. L. FIOROTTO, and F. W. BOOTH. Insulin-like growth factor-I extends in vitro replicative life span of skeletal muscle satellite cells by enhancing G1/S cell cycle progression via the activation of phosphatidylinositol 3'-kinase/Akt signaling pathway. *J. Biol. Chem.* 275:35942–35952, 2000.
18. CHAKRAVARTHY, M. V., B. S. DAVIS, and F. W. BOOTH. IGF-I restores satellite cell proliferative potential in immobilized old skeletal muscle. *J. Appl. Physiol.* 89:1365–1379, 2000.
19. CHILDS, T. E., E. E. SPANGENBURG, D. R. VYAS, and F. W. BOOTH. Sequential alterations in protein signaling cascades during recovery from muscle atrophy. *Am. J. Physiol. Cell Physiol.* 285:C391–C398, 2003.
20. DALLA LIBERA, L., R. SABBADINI, C. RENKEN, et al. Apoptosis in the skeletal muscle of rats with heart failure is associated with increased serum levels of TNF-alpha and sphingosine. *J Mol. Cell Cardiol.* 33:1871–1878, 2001.
21. DARR, K. C., and E. SCHULTZ. Exercise-induced satellite cell activation in growing and mature skeletal muscle. *J. Appl. Physiol.* 63:1816–1821, 1987.
22. DARR, K. C., and E. SCHULTZ. Hindlimb suspension suppresses muscle growth and satellite cell proliferation. *J. Appl. Physiol.* 67:1827–1834, 1989.
23. EDGERTON, V. R., R. R. ROY, D. L. ALLEN, and R. J. MONTI. Adaptations in skeletal muscle disuse or decreased-use atrophy. *Am. J. Phys. Med. Rehabil.* 81(Suppl. 11):S127–S147, 2002.
24. EDGERTON, V. R., M. Y. ZHOU, Y. OHIRA, et al. Human fiber size and enzymatic properties after 5 and 11 days of spaceflight. *J. Appl. Physiol.* 78:1733–1739, 1995.
25. FISCHBACH, G. D., and N. ROBBINS. Changes in contractile properties of disused soleus muscles. *J. Physiol.* 201:305–320, 1969.
26. FITTS, R. H., D. R. RILEY, and J. J. WIDRICK. Functional and structural adaptations of skeletal muscle to microgravity. *J. Exp. Biol.* 204:3201–3208, 2001.
27. FRIDAY, B. B., V. HORSLEY, and G. K. PAVLATH. Calcineurin activity is required for the initiation of skeletal muscle differentiation. *J. Cell Biol.* 149:657–666, 2000.
28. GOLDSPINK, D. F., A. J. MORTON, P. LOUGHNA, and G. GOLDSPINK. The effect of hypokinesia and hypodynamia on protein turnover and the growth of four skeletal muscles of the rat. *Pflügers Arch.* 407:333–340, 1986.
29. GOMES, M. D., S. H. LECKER, R. T. JAGOE, A. NAVON, and A. L. GOLDBERG. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proc. Natl. Acad. Sci. USA* 98: 14440–14445, 2001.
30. HAWKE, T. J., and D. J. GARRY. Myogenic satellite cells: physiology to molecular biology. *J. Appl. Physiol.* 91:534–551, 2001.
31. HIKIDA, R. S., S. VAN NOSTRAN, J. D. MURRAY, R. S. STARON, S. E. GORDON, and W. J. KRAEMER. Myonuclear loss in atrophied soleus muscle fibers. *Anat. Rec.* 247:350–354, 1997.
32. HNIK, P., R. VEJSADA, D. F. GOLDSPINK, S. KASICKI, and I. KREKULE. Quantitative evaluation of electromyogram activity in rat extensor and flexor muscles immobilized at different lengths. *Exp. Neurol.* 88:515–528, 1985.
33. HUNTER, R. B., E. STEVENSON, A. KONCAREVIC, H. MITCHELL-FELTON, D. A. ESSIG, and S. C. KANDARIAN. Activation of an alternative NF-kappaB pathway in skeletal muscle during disuse atrophy. *FASEB J.* 16:529–538, 2002.
34. HUSMANN, I., L. SOULET, J. GAUTRON, I. MARTELLY, and D. BARRITAULT. Growth factors in skeletal muscle regeneration. *Cytokine Growth Factor Rev.* 7:249–258, 1996.
35. INGBER, D. E. Tensegrity: the architectural basis of cellular mechanotransduction. *Annu. Rev. Physiol.* 59:575–599, 1997.
36. JAGOE, R. T., S. H LECKER, M. GOMES, and A. L. GOLDBERG. Patterns of gene expression in atrophying skeletal muscles: response to food deprivation. *FASEB J.* 16:1697–1712, 2002.
37. JENNISCHE, E., A. SKOTTNER, and H. A. HANSSON. Satellite cells express the trophic factor IGF-I in regenerating skeletal muscle. *Acta Physiol. Scand.* 129:9–15, 1987.
38. KASPER, C. E. Sarcolemmal disruption in reloaded atrophic skeletal muscle. *J. Appl. Physiol.* 79:607–614, 1995.
39. KASPER, C. E., and L. XUN. Cytoplasm-to-myonucleus ratios in plantaris and soleus muscle fibres following hindlimb suspension. *J. Muscle Res. Cell Motil.* 17:603–610, 1996.
40. KELLY, A. E. Satellite cells and myofiber growth in the rat soleus and extensor digitorum longus muscles. *Dev. Biol.* 65:1–10, 1978.
41. KONDO, H., J. KODAMA, T. KISHIBE, and Y. ITOKAWA. Oxidative stress during recovery from muscle atrophy. *FEBS Lett.* 326:189–191, 1993.
42. KONDO, H., I. NAKAGAKI, S. SASAKI, S. HORI, and Y. ITOKAWA. Mechanism of oxidative stress in skeletal muscle atrophied by immobilization. *Am. J. Physiol.* 265:E839–E844, 1993.
43. KRIPPENDORF, B. B., and D. A. RILEY. Distinguishing unloading-versus reloading-induced changes in rat soleus muscle. *Muscle Nerve* 16:99–108, 1993.
44. LLOVERA, M., C. GARCIA-MARTINEZ, N. AGELL, F. J. LOPEZ-SORIANO, and J. M. ARGILES. TNF can directly induce the expression of ubiquitin-dependent proteolytic system in rat soleus muscles. *Biochem. Biophys. Res. Commun.* 230:238–241, 1997.
45. METTER, E. J., L. A. TALBOT, M. SCHRAGER, and R. CONWIT. Skeletal muscle strength as a predictor of all-cause mortality in healthy men. *J. Gerontol. A Biol. Sci. Med. Sci.* 57:B359–B365, 2002.
46. MITCHELL, P. O., S. T. MILLS, and G. K. PAVLATH. Calcineurin differentially regulates maintenance and growth of phenotypically distinct muscles. *Am. J. Physiol. Cell Physiol.* 282:C984–C992, 2002.
47. MITCHELL, P. O., and G. K. PAVLATH. A muscle precursor cell-dependent pathway contributes to muscle growth after atrophy. *Am. J. Physiol. Cell Physiol.* 281:C1706–C1715, 2001.
48. MOREY-HOLTON, E. R., and R. K. GLOBUS. Hindlimb unloading rodent model: technical aspects. *J. Appl. Physiol.* 92:1367–1377, 2002.
49. MORRISON, P. R., G. W. MULLER, and F. W. BOOTH. Actin synthesis rate and mRNA level increase during early recovery of atrophied muscle. *Am. J. Physiol.* 253:C205–C209, 1987.
50. MORRISON, P. R., J. A. MONTGOMERY, T. S. WONG, and F. W. BOOTH. Cytochrome c protein-synthesis rates and mRNA contents during atrophy and recovery in skeletal muscle. *Biochem. J.* 241: 257–263, 1987.
51. MOZDZIAK, P. E., P. M. PULVERMACHER, and E. SCHULTZ. Unloading of juvenile muscle results in a reduced muscle size 9 wk after reloading. *J. Appl. Physiol.* 88:158–164, 2000.
52. MOZDZIAK, P. E., E. SCHULTZ, and R. G. CASSENS. Myonuclear accretion is a major determinant of avian skeletal muscle growth. *Am. J. Physiol.* 272:C565–571, 1997.
53. REARDON, K., M. GALEA, X. DENNETT, P. CHOONG, and E. BYRNE. Quadriceps muscle wasting persists 5 months after total hip arthroplasty for osteoarthritis of the hip: a pilot study. *Intern. Med. J.* 31:7–14, 2001.
54. REID, M. B., and Y. P. LI. Cytokines and oxidative signalling in skeletal muscle. *Acta. Physiol. Scand.* 171:225–232, 2001.

55. RILEY, D. A., E. I. ILYINA-KAKUEVA, S. ELLIS, J. L. BAIN, G. R. SLOCUM, and F. R. SEDLAK. Skeletal muscle fiber, nerve, and blood vessel breakdown in space-flown rats. *FASEB J.* 4:84–91, 1990.
56. ROSENBLATT, J. D., D. YONG, and D. J. PARRY. Satellite cell activity is required for hypertrophy of overloaded adult rat muscle. *Muscle Nerve* 17:608–613, 1994.
57. SAKUMA, K, J. NISHIKAWA, R. NAKAO, et al. Calcineurin is a potent regulator for skeletal muscle regeneration by association with NFATc1 and GATA-2. *Acta Neuropathol. (Berl.)* 105:271–280, 2003.
58. SAKUMA, K., K. WATANABE, M. SANO, I. URAMOTO, and T. TOTSUKA. Differential adaptation of growth and differentiation factor 8/myostatin, fibroblast growth factor 6 a, fibroblast growth factor 6 and leukemia inhibitory factor in overloaded, regenerating and denervated rat muscles. *Biochim. Biophys. Acta* 1497:77–88, 2000.
59. SCHIAFFINO, S., S. P. BORMIOLI, and M. ALOISI. Cell proliferation in rat skeletal muscle during early stages of compensatory hypertrophy. *Virchows Arch. B Cell Pathol.* 11:268–273, 1972.
60. SCHIAFFINO, S., S. P. BORMIOLI, and M. ALOISI. The fate of newly formed satellite cells during compensatory muscle hypertrophy. *Virchows Arch. B Cell Pathol.* 21:113–118, 1976.
61. SCHULTZ, E., K. C. DARR, and A. MACIUS. Acute effects of hindlimb unweighting on satellite cells of growing skeletal muscle. *J. Appl. Physiol.* 76:266–270, 1994.
62. SCHULTZ, E., and B. H. LIPTON. Skeletal muscle satellite cells: changes in proliferation potential as a function of age. *Mech. Ageing Dev*. 20:377–383, 1982.
63. SPANGENBURG, E. E., and F. W. BOOTH. Multiple signaling pathways mediate LIF-induced skeletal muscle satellite cell proliferation. *Am. J. Physiol. Cell Physiol.* 283:C204–C211, 2002.
64. STRAWBRIDGE, W. J., S. J. SHEMA, J. L. BALFOUR, H. R. HIGBY, and G. A. Kaplan. Antecedents of frailty over three decades in an older cohort. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 53:S9–S16, 1998.
65. ST PIERRE, B. A., and J. G. TIDBALL. Differential response of macrophage subpopulations to soleus muscle reloading after rat hindlimb suspension. *J. Appl. Physiol*. 77:290–297, 1994.
66. THOMASON, D. B., R. B. BIGGS, and F. W. BOOTH. Protein metabolism and β-myosin heavy-chain mRNA in unweighted soleus muscle. *Am. J. Physiol.* 257:R300–R305, 1989.
67. THOMASON, D. B., and F. W. BOOTH. Atrophy of the soleus muscle by hindlimb unweighting. *J. Appl. Physiol.* 68:1–12, 1990.
68. TUCKER, K. R., M. J. SEIDER, and F. W. BOOTH. Protein synthesis rates in atrophied gastrocnemius muscles after limb immobilization. *J. Appl. Physiol.* 51:73–77, 1981.
69. VIJAYAN, K., J. L. THOMPSON, and D. A. RILEY. Sarcomere lesion damage occurs mainly in slow fibers of reloaded rat adductor longus muscles. *J. Appl. Physiol.* 85:1017–1023, 1998.
70. WATSON, P. A., J. P. STEIN, and F. W. BOOTH. Changes in actin synthesis and α-actin-mRNA content in rat muscle during immobilization. *Am. J. Physiol.* 247:C39–C44, 1984.
71. WONG, T. S., and F. W. BOOTH. Protein metabolism in rat gastrocnemius muscle after stimulated chronic concentric exercise. *J. Appl. Physiol.* 69:1709–1717, 1990.
72. WONG, T. S., and F. W. BOOTH. Protein metabolism in rat tibialis anterior muscle after stimulated chronic eccentric exercise. *J. Appl. Physiol.* 69:1718–1724, 1990.
73. ZARZHEVSKY, N., E. CARMELI, D. FUCHS, R. COLEMAN, H. STEIN, and A. Z. REZNICK. Recovery of muscles of old rats after hindlimb immobilisation by external fixation is impaired compared with those of young rats. *Exp. Gerontol.* 36:125–140, 2001.