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Muscle Stem Cells and Exercise Training

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HAWKE, T.J. Muscle Stem Cells and Exercise Training. *Exerc. Sport Sci. Rev.*, Vol. 33, No. 2, pp. 63–68, 2005. *Adult skeletal muscle fibers are terminally differentiated such that hypertrophy and regeneration require resident stem cell populations. This review examines the current understanding of the factors regulating muscle stem cells in response to exercise and identify the role of these cells in the adaptive response of skeletal muscle to endurance and resistance exercise training.* **Key Words:** satellite cells, SP cells, muscle regeneration, injury, growth factors, hypertrophy

INTRODUCTION

Although endurance training is associated with high-repetition–low-resistance exercise, significant muscle damage can occur if the duration or mode of exercise is extreme. For example, both marathon running and downhill running (eccentric exercise) can lead to significant muscle fiber damage, and many studies use eccentric exercise as a modality to induce muscle injury.

In contrast to endurance training, resistance exercise training is associated with high-intensity–low-repetition workloads leading to increases in muscular strength, power, and oxidative capacity, with little change in aerobic capacity. The workloads placed on skeletal muscle during resistance training are at or near maximal capacity, and as such produce significant perturbations to the skeletal muscle fibers and the associated extracellular matrix.

The muscle damage associated with intense exercise (regardless of exercise type) includes disruption to the extracellular matrix, basal lamina, and sarcolemma, resulting in the release of intracellular proteins such as myoglobin and creatine kinase. Within the muscle fiber, damage to contractile and cytoskeletal proteins can also occur, leading to decreased fiber tension and even death of the muscle fiber. Interestingly, whereas both endurance and resistance exercise can result in muscle injury, resistance training is more likely to be associated with increases in fiber cross-sectional area. The reasons for this point to differences in the integration of hormonal, metabolic, mechanical, neuronal, and immune responses, which are all likely involved in the distinct transcriptional responses that characterize endurance and resistance training.

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RESIDENT MUSCLE STEM CELL POPULATIONS

Adult skeletal muscle contains identifiable cell populations with stem cell–like characteristics. One of these cell populations, the myogenic progenitor cells (MPCs), are also known as satellite cells based on their location at the periphery of adult myofibers (3). These undifferentiated progenitor cells are the most thoroughly characterized of the resident muscle stem cell populations. The MPCs are quiescent in the unstressed muscle, but can reenter the cell cycle (become "activated") in response to signals associated with muscle damage. After activation, these cells will proliferate and migrate to the site of injury to repair or replace damaged myofibers by fusing together and/or fusing to existing myofibers (Fig. 1A) (6).

The fusion of MPCs to existing myofibers is critical for large increases in myofiber cross-sectional area, and works on the premise of the myonuclear domain theory. This theory suggests that the myonucleus controls the production of mRNA and proteins for a finite volume of cytoplasm, such that increases in fiber size (hypertrophy) must be associated with a proportional increase in myonuclei, which are contributed from the MPC population (Fig. 1B). Importantly, the MPCs are self-renewing, such that a residual pool of these cells is reestablished after each discrete episode of muscle injury, and therefore capable of supporting additional rounds of regeneration. Whereas the MPC displays some similarities to other adult stem cell populations (such as self-renewal and a limited

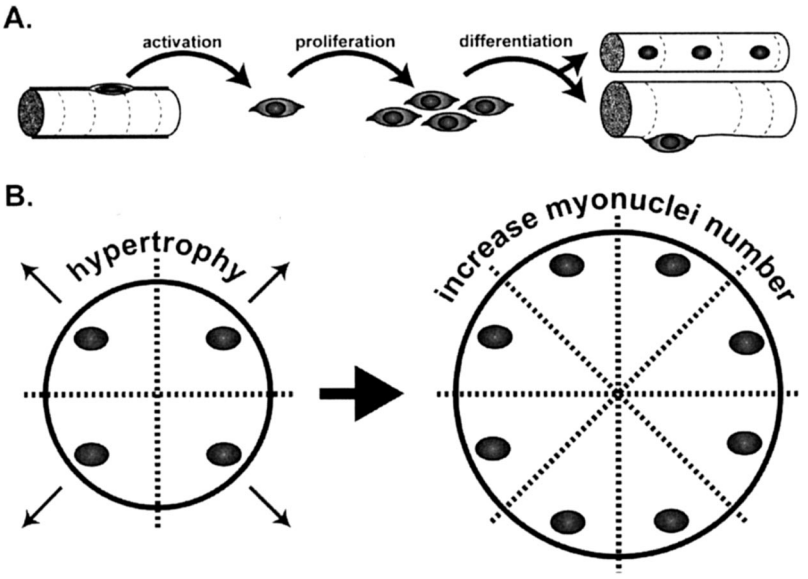

Figure 1. Myogenic progenitor cells mediate skeletal muscle regeneration and hypertrophy. (A) Quiescent myogenic progenitor cells (MPCs) reside in a peripheral location on the mature myofiber. In response to muscle damage, these cells will become activated, proliferate, and migrate to the site of injury. If the myofibers are extensively damaged, the MPCs will differentiate and fuse together to generate a new myofiber. Newly regenerated myofibers are identifiable based on their centrally located nuclei (top fiber). In response to hypertrophic stimuli, the MPCs will differentiate and fuse to existing myofibers, essentially donating their nuclei (bottom fiber). (B) The myonuclear domain theory suggests that the volume of cytoplasm "managed" by a nucleus within a myofiber is finite, such that any increases in myofiber cross-sectional area (hypertrophy) must be associated with a proportional increase in myonuclei. Evidence to date indicates that fusion of MPCs with the myofiber is responsible for the increase in myonuclei with hypertrophy. Note that the cross-sectional area within each of the triangles of the myofibers is similar.

capacity to adopt alternative lineages), it is largely assumed that these cells are "committed" to the skeletal muscle lineage.

An additional population of cells with stem cell–like characteristics has recently been identified within numerous adult tissues, including skeletal muscle. These muscle stem cells can be isolated using dual-wavelength flow cytometric analysis (FACS) based on their ability to efflux the DNA dye, Hoechst 33342. Muscle stem cells isolated using FACS analyses are termed SP cells because they appear as a "side population" on the FACS profile (Fig. 2A). Muscle SP cells are far rarer than the MPCs within resting adult skeletal muscle (<0.2 vs 2–5% of all muscle nuclei), and have been shown to display a greater ability to adopt other cell lineages (plasticity) than the MPC population (7,11).

A number of studies have recently identified subpopulations within the muscle SP cell populations that display differential capacities for self-renewal, plasticity, proliferation, and differentiation (4). Whether these subpopulations

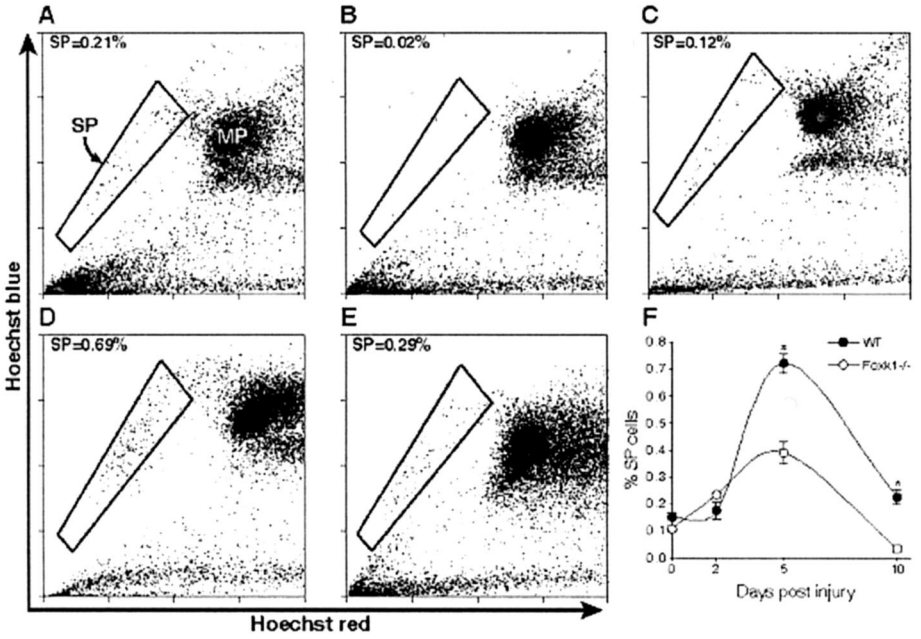

Figure 2. Muscle SP cells increase after injury, but are decreased in Foxk1 mutant skeletal muscle. (A) Representative FACS profile of muscle SP cells. Note that the SP cells are located in the gated region and account for 0.21% of the total cell population. (B) Inhibition of the SP cell phenotype after the addition of the Abcg2 inhibitor, FTC. (C) FACS profile reveals fewer SP cells in the Foxk1 mutant muscle compared to wild-type skeletal muscle. (D) Increased SP cell numbers (compared to uninjured skeletal muscle in panel A) are observed 5 d after injury of wild-type skeletal muscle. (E) Increased SP cell numbers (compared to uninjured Foxk1 mutant skeletal muscle) 5 d after cardiotoxin injury in Foxk1 mutant skeletal muscle. Note the increase in SP cell numbers in injured Foxk1 mutant skeletal muscle is less than injured wild-type skeletal muscle. (F) Quantitation of the SP cell numbers in wild-type and Foxk1 mutant injured skeletal muscle. Note that at each time period, wild-type skeletal muscle has increased numbers of SP cells (mean ± SEM). (Reprinted from Meeson, A.P., T.J. Hawke, S. Graham, N. Jiang, J. Eltermann, K. Hutcheson, J.M. DiMaio, T. Gallardo and D.J. Garry. Cellular and molecular regulation of skeletal muscle SP cells. *Stem Cells* 22:1305–1320, 2004. Copyright © 2004 AlphaMed Press 1066-6099. Used with permission.)

represent distinct cell populations, or whether they are the same cell population adapting to changes in their microenvironment, is still under investigation.

ARE MUSCLE SP CELLS INVOLVED IN SKELETAL MUSCLE REGENERATION?

Virtually all mammalian tissues have been demonstrated to contain a SP cell complement. A complete discussion of the contribution and potential role of these nonmuscle SP cells in skeletal muscle regeneration is beyond the scope of this review; the reader is referred to Charge and Rudnicki (2) and Grounds *et al.* (4) for a review of the potential contribution of these other stem cell populations in muscle repair.

Skeletal muscle hypertrophy and regeneration were previously believed to be mediated solely through the MPCs. There is increasing evidence, however, to suggest that, given some circumstances, muscle (and even nonmuscle) SP cell populations can contribute to the regeneration of injured skeletal muscle (Fig. 3). For example, Gussoni *et al.* (5) injected muscle SP cells into the tail vein of lethally irradiated myopathic (*mdx*) mice and demonstrated the ability of a small number of these cells (<0.5%) to contribute to regenerated muscle, and also found evidence to suggest that transplanted muscle SP cells may give rise to quiescent MPCs. Consistent with these findings, an intriguing study by LaBarge and Blau (9) found that bone marrow–derived SP cells could contribute to skeletal muscle repair in lethally irradiated mice. Furthermore, these transplanted cells gave rise to quiescent MPCs that were capable of contributing to the formation of approximately 3.5% of the regenerated myofibers in response to subsequent exercise-induced damage.

Anecdotal support for the role of muscle SP cells in skeletal muscle repair comes from the finding that muscle SP cell number increases significantly in response to muscle injury (Fig. 2). Furthermore, in a mouse model with impaired muscle regenerative capacity (Foxk1 mutant mice), there is a reduction in their muscle SP cell number, and the injury-induced increase in the SP cell number is significantly blunted (Fig. 2) (11). Additionally, Rehman *et al.* (12) demonstrated an increase in circulating endothelial progenitor cells with a single bout of exhaustive exercise, leading the authors to speculate that these cells were mobilized for the purpose of repair and angiogenesis in response to intense exercise. This finding is in agreement with the increase in muscle SP cell number with muscle injury, which may be mobilized for the purpose of contributing to skeletal muscle regeneration (11).

Taken together, these studies provide some support for the hypothesis that the SP cell population may be precursors to the MPCs and, given the appropriate extracellular milieu and cell–cell interactions, that SP cells are capable of adopting a myogenic lineage and contributing to skeletal muscle regeneration. However, it has yet to be elucidated whether endogenous SP cells, particularly muscle SP cells, are involved in the hypertrophy or exercise-induced regeneration of normal skeletal muscle. To date, the contribution of endogenous SP cells to these processes appears minimal, although from a therapeutic perspective, lethal irradiation followed by significant muscle injury, or increasing local growth factor levels, may increase their overall contribution (4).

EXTRINSIC CUES REGULATING MUSCLE STEM CELLS DURING REGENERATION AND HYPERTROPHY

The ability of skeletal muscle to respond to stressors such as strenuous exercise is mediated by a complex array of extrinsic and intrinsic cues. A critical step in this adaptability involves modulating the timing, availability, and receptor density for the muscle growth factors. Although there are obvious physiological, cellular, and molecular distinctions between skeletal muscle regeneration and hypertrophy, both processes share similarities regarding MPC activation, proliferation, and differentiation. With skeletal muscle hypertrophy, the MPCs will fuse to the existing myofibers, essentially

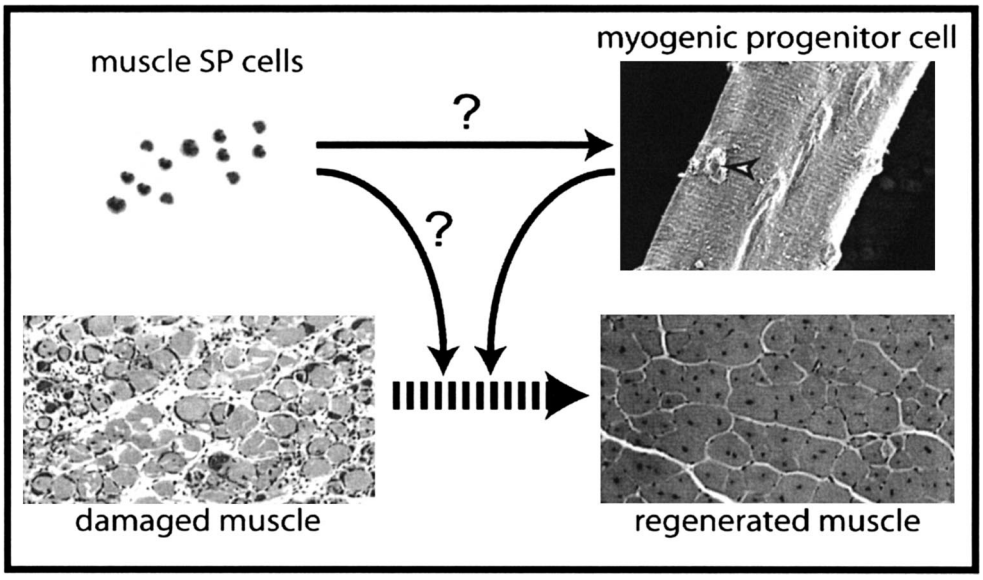

Figure 3. Role of muscle stem cell populations in regenerating muscle. There has been a tremendous amount of interest in investigating the contribution of various muscle stem cell populations in regenerating skeletal muscle. It is well established that the myogenic progenitor cells are largely responsible for the repair that occurs. The role of another muscle stem cell population, the muscle SP cells, in the repair process is currently unknown. Recent studies suggest that in certain circumstances, the muscle SP cells can aid in the regeneration of muscle. Also of interest is the possibility that muscle SP cells may be the precursor population for the myogenic progenitor cells.

donating their nuclei, whereas regeneration from more extensive muscle damage may result in the fusing together of MPCs to generate a new myofiber. Newly generated myofibers are identified by their centrally located nuclei (Figs. 1 and 4).

Because the role and regulation of the muscle SP cell population are still largely unknown, this section focuses on the MPC and its role in the adaptability of skeletal muscle to exercise.

Activation

The activation of MPCs is characterized by alterations in their morphology (increased cytoplasmic to nuclear ratio, increased cytoplasmic organelles, and reduced heterochromatin) and their adhesion characteristics to the mature myofiber. A potential mechanical link between myofiber damage and MPC activation has recently been uncovered (14). In this model, myofiber damage leads to the bolus release of nitric oxide, which mediates the release of active hepatocyte growth factor from its heparin sulfate chains on the extracellular matrix and surrounding myofibers. The release of hepatocyte growth factor in response to muscle injury occurs rapidly (order of minutes), and is proportional to the degree of muscle injury. Hepatocyte growth factor binding to its receptor, c-met, located on the MPC plasma membrane, is one of the earliest events in MPC activation, and leads to a cascade of signaling events promoting cell proliferation and changes in focal adhesion (Fig. 5).

Recently, an isoform of insulin-like growth factor, termed mechanogrowth factor, has been identified; it is released early (minutes to hours) after increased loading or stretch of the muscle (1). The role played by mechanogrowth factor in MPC activation is currently being investigated, but based on its early release, it may be involved in promoting transcriptional changes associated with preparing the cell for increased cellular proliferation.

Proliferation and Differentiation

There are numerous studies demonstrating the potential involvement of various growth factors in regulating the MPCs *in vitro* (6). Although these *in vitro* studies do provide insight into the regulation of the MPC, the effects observed are not always consistent with *in vivo* studies. These differences may be attributed to variations in growth factor timing, availability, and receptor density.

The degree of immune response associated with strenuous exercise is proportional to the degree of mechanical damage to the muscle; endurance exercise is associated with a more systemic immune response, whereas resistance exercise is associated with a more localized immune response. Within a few hours after skeletal muscle damage, circulating neutrophils are increased and the damaged tissue attracts macrophages, which respond by releasing chemokines and cytokines that will act as an attractant for the migration of neutrophils, monocytes, and MPCs, as well as increase blood vessel permeability, allowing for an increase in fluid and protein transition into the extracellular space. Among the cytokines associated with the inflammatory response, leukemia inhibitory factor has been demonstrated to increase significantly in response to exercise-induced injury, and is capable of increasing MPC proliferation *in vitro*. Interestingly, the exogenous administration of leukemia inhibitory factor to skeletal muscle can promote MPC proliferation, myofiber hypertrophy, and improved regeneration after injury. Collectively, the inflammatory cytokines, and in particular the IL-6 family of cytokines (of which leukemia inhibitory factor is a member), appear to play an integral role in the repair process after myotrauma (15).

Of all the muscle growth factors, insulin-like growth factor-I (IGF-I) has been the most thoroughly characterized during muscle hypertrophy. In response to resistance training, IGF-I secretion from skeletal muscle is elevated, and

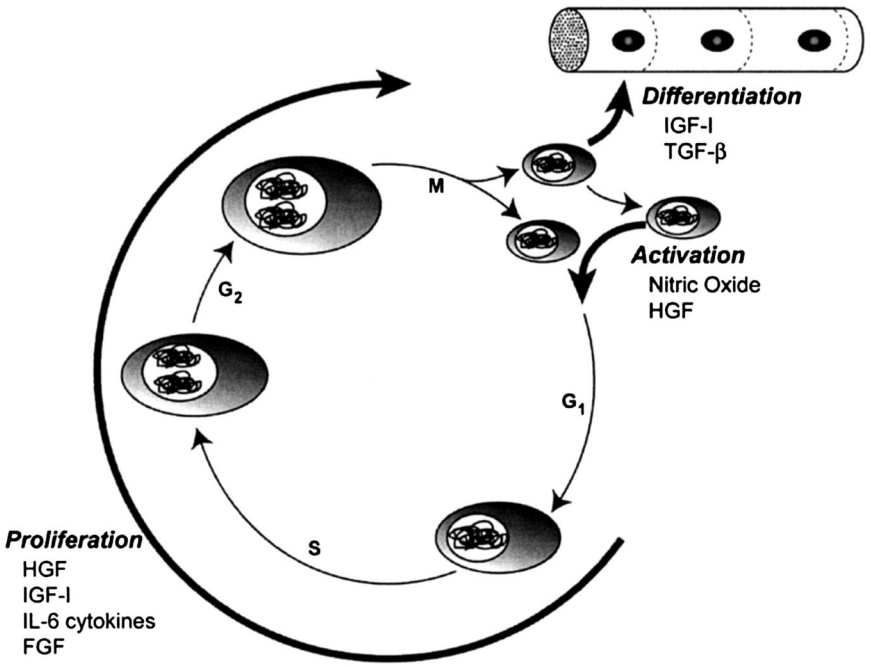

Figure 4. Extrinsic cues regulating the cell-cycle progression of muscle progenitor cells. In response to stressors such as myotrauma, quiescent myogenic progenitor cells become activated, undergo proliferation, and ultimately differentiate to produce new muscle fibers. These newly regenerated fibers can be identified by their centrally located nuclei. Numerous growth factors have been shown to be important in mediating the progression of the myogenic progenitor cells through these particular phases. This schematic outlines only a few of the growth factors known to modulate myogenic progenitor cell activity. HGF, hepatocyte growth factor; IGF-I, insulin-like growth factor-I; FGF, fibroblast growth factor; TGF-β, transforming growth factor-β.

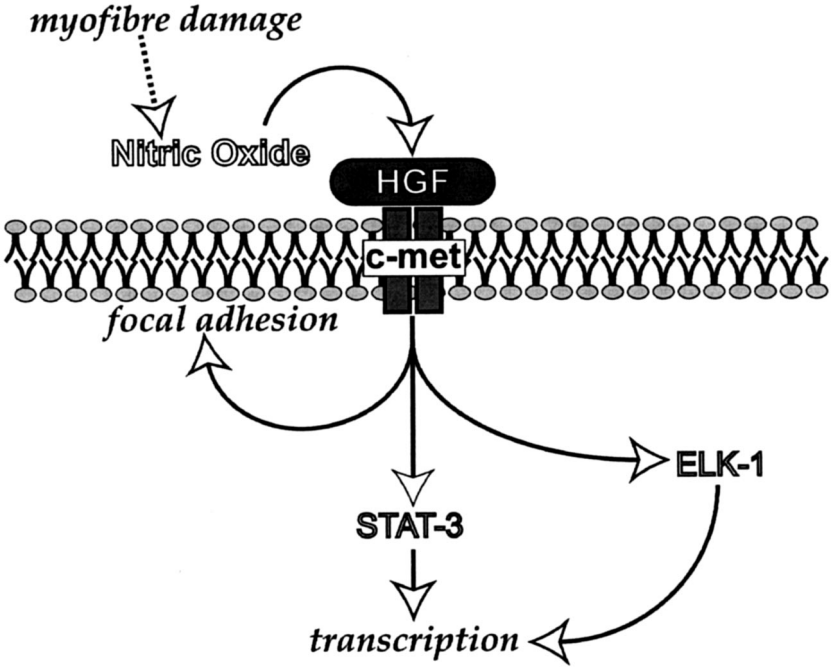

Figure 5. MPC activation is mediated through nitric oxide and hepatocyte growth factor. Damage to the mature myofiber leads to a bolus release of nitric oxide, which mediates the release of active hepatocyte growth factor (HGF) from the extracellular matrix and mature myofibers (14). Once released, HGF will bind to its receptor (c-met) located on the plasma membrane of the myogenic progenitor cell. This interaction initiates a cascade of signaling events promoting the transcription of early-response genes and key cell-cycle regulatory genes. In addition, activation of the c-met receptor by HGF can lead to changes in the adhesion characteristics of the myogenic progenitor cell.

promotes proliferation and fusion of the MPCs. The ability for IGF-I to mediate both MPC proliferation and differentiation may be caused by the capacity to signal through two distinct signaling cascades. The proliferative effects of IGF-I have been largely attributed to the Ras/Raf/MEK/ERK pathway, whereas the PI3K/AKT signaling pathway has been proposed to mediate the differentiation cues (10). Although cross-talk between these two cascades is likely, recent evidence has demonstrated a key role of the PI3K/AKT pathway in mediating MPC proliferation (10). The essential role played by IGF-I in MPC regulation has been demonstrated *in vivo*, where superfusion or overexpression of IGF-I results in skeletal muscle hypertrophy and prevention of age-related sarcopenia 1.

In general, the transforming growth factor (TGF)-β family (including myostatin) of cytokines is proposed to inhibit MPC proliferation and differentiation, primarily through silencing the transcriptional activation of the MyoD family members. In response to muscle damage, circulating and local TGF-β levels are elevated. The inhibition of MPC proliferation and differentiation by TGF-β in response to muscle damage may seem counterintuitive. Sakuma *et al.* (13) suggest that during regeneration from muscle injury, TGF-βII ligand and receptor levels are reciprocally expressed, and that this reciprocal expression pattern may result in the initial promotion of cellular proliferation followed by enhanced muscle differentiation. In addition to modulating MPC activity, they are involved in promoting normal skeletal muscle architecture by regulating local collagen synthesis in tendon-related connective tissue (13).

CHANGES IN EXTRINSIC CUES WITH CHRONIC TRAINING

As stated previously, both endurance and resistance exercise result in varying degrees of muscle fiber damage. In response to the chronic perturbations placed on the skeletal muscles during resistance training, there are significant increases in fiber size, myonuclei, and MPC number (8). These alterations are not observed with chronic endurance exercise training, although significant myofiber turnover can still occur (3). This dichotomy could be explained, in part, by the observation that elevations in plasma testosterone and growth hormone levels appear to be more dependent on exercise intensity than exercise volume. Increases in these hormones may mediate increases in IGF-I, resulting in the elevated MPC number and myofiber hypertrophy observed with chronic resistance training or exogenous testosterone administration.

Despite the tremendous interest in hormones, growth factors, and exercise, there is limited information on changes in these factors in response to chronic training. Furthermore, although changes in circulating hormone levels may occur in response to either resistance or endurance training, it is also important to identify whether reciprocal changes in receptor density occurs. Also of interest is the role and regulation of the extracellular matrix with chronic training. For example, does the extracellular matrix become more or less sensitive to muscle damage with training?

SUMMARY

The adaptability of skeletal muscle in response to a range of stressors is remarkable. Although we have learned much about the general physiology of this organ system, our understanding of the mechanisms regulating this adaptability remains a fertile area of research.

Acknowledgments

The author recognizes the research efforts of those included and omitted (only because of space considerations) who have contributed to this field of

study. In some cases, review articles have been used to direct the reader to original articles or more comprehensive summaries of the literature. A special thanks to Drs. R. Allen, F. Booth, S. Kanatous, and A. Meeson for helpful discussions and critical reviews of this manuscript. The author's work is supported by grants from the Canadian Foundation for Innovation, the Ontario Innovations Trust, and the Hospital for Sick Kids Foundation.

References

1. Adams, G.R. Invited Review: Autocrine/paracrine IGF-I and skeletal muscle adaptation. *J. Appl. Physiol.* 93:1159–1167, 2002.
2. Charge, S.B. and M.A. Rudnicki. Cellular and molecular regulation of muscle regeneration. *Physiol. Rev.* 84:209–238, 2004.
3. Collins, M., V. Renault, L.A. Grobler, A. St Clair Gibson, M.I. Lambert, E. Wayne Derman, G.S. Butler-Browne, T.D. Noakes and V. Mouly. Athletes with exercise-associated fatigue have abnormally short muscle DNA telomeres. *Med. Sci. Sports Exerc.* 35:1524–1528, 2003.
4. Grounds, M.D., J.D. White, N. Rosenthal and M.A. Bogoyevitch. The role of stem cells in skeletal and cardiac muscle repair. *J. Histochem. Cytochem.* 50:589–610, 2002.
5. Gussoni, E., Y. Soneoka, C.D. Strickland, E.A. Buzney, M.K. Khan, A.F. Flint, L.M. Kunkel and R.C. Mulligan. Dystrophin expression in the *mdx* mouse restored by stem cell transplantation. *Nature* 401:390–394, 1999.
6. Hawke, T.J. and D.J. Garry. Myogenic satellite cells: physiology to molecular biology. *J. Appl. Physiol.* 91:534–551, 2001.
7. Hawke, T.J., A.P. Meeson, N. Jiang, S. Graham, K. Hutcheson, J.M. DiMaio, and D.J. Garry. p21 is essential for normal myogenic progenitor cell function in regenerating skeletal muscle. *Am. J. Physiol.* 285:C1019-C1027, 2003.
8. Kadi, F. and L.E. Thornell. Concomitant increases in myonuclear and satellite cell content in female trapezius muscle following strength training. *Histochem. Cell Biol.* 113:99–103, 2000.
9. LaBarge, M.A. and H.M. Blau. Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. *Cell* 111:589–601, 2002.
10. Machida, S. and F.W. Booth. Insulin-like growth factor 1 and muscle growth: implication for satellite cell proliferation. *Proc. Nutr. Soc.* 63:337–340, 2004.
11. Meeson, A.P., T.J. Hawke, S. Graham, N. Jiang, J. Eltermann, K. Hutcheson, J.M. DiMaio, T. Gallardo and D.J. Garry. Cellular and molecular regulation of skeletal muscle SP cells. *Stem Cells* 22:1305–1320, 2004.
12. Rehman, J., J. Li, L. Parvathaneni, G. Karlsson, V.R. Panchal, C.J. Temm, J. Mahenthiran and K.L. March. Exercise acutely increases circulating endothelial progenitor cells and monocyte-/macrophage-derived angiogenic cells. *J. Am. Coll. Cardiol.* 43:2314–2318, 2004.
13. Sakuma, K., K. Watanabe, M. Sano, S. Kitajima, K. Sakamoto, I. Uramoto, and T. Totsuka. The adaptive response of transforming growth factor-beta 2 and -beta RII in the overloaded, regenerating and denervated muscles of rats. *Acta. Neuropathol. (Berl).* 99:177–85, 2000.
14. Tatsumi, R., A. Hattori, Y. Ikeuchi, J.E. Anderson and R.E. Allen. Release of hepatocyte growth factor from mechanically stretched skeletal muscle satellite cells and role of pH and nitric oxide. *Mol. Biol. Cell* 13:2909–2918, 2002.
15. Vierck, J., B. O'Reilly, K. Hossner, J. Antonio, K. Byrne, L. Bucci and M. Dodson, Satellite cell regulation following myotrauma caused by resistance exercise. *Cell Biol. Int.* 24:263–272, 2000.