Exercise-enhanced satellite cell proliferation and new myonuclear accretion in rat skeletal muscle

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A single bout of strenuous or unaccustomed exercise induces histological evidence of segmental injury and subsequent regeneration in a small percentage of fibers (2, 33), and a latent increase in the activation and proliferation of satellite cells within both slow- and fast-twitch muscles of adult rats (8, 15). The satellite cells are mononucleated myogenic precursors that aid in the repair or replacement of injured or necrotic muscle fibers (1, 5) and can also function as a source of the maturation of newly formed myofibers. Because satellite cell progeny fuse with existing fibers, the cumulative increase or accretion of such newly produced myonuclei would augment the DNA available to aid in fiber regeneration and maturation and possibly other adaptive processes. However, the effects of functional loading, beyond that of normal activity levels, on satellite cell proliferation and the accretion of newly produced myonuclei during the early regeneration of adult muscle after exercise-induced injury has not, to our knowledge, been determined.

We hypothesized that continued overloading by daily treadmill exercise, compared with normal activity alone, would enhance satellite cell activation and new myonuclear accretion in skeletal muscle after exercise-induced injury.

The aim of the present study was to determine the effects of either one or daily bouts of locomotory exercise on satellite cell proliferation and new myonuclear accretion in hindlimb muscles of previously untrained, adult rats. Our results demonstrated that continuation...
of the exercise bouts that initially induced focal muscle injury, compared with normal weight-bearing activity alone, resulted in an increase in satellite cell proliferative activity and a greater accretion of new myonuclei during the early phase of muscle fiber repair and regeneration. Observed coincident with a lower incidence of histological fiber injury and unchanged fiber size and myonuclear number, the new myonuclei likely contributed to an exercise-enhanced repair, regeneration, or regrowth of fibers during this time.

METHODS

A total of 64 female Wistar rats (10–12 wk old, 246–306 g) were used in this study. Rats were housed in a temperature-controlled room maintained on a 12:12-h light-dark cycle with food pellets and water available ad libitum. All procedures for animal care and treatment were in accordance with guidelines of the Canadian Council on Animal Care and approved by the University of Toronto Animal Care Committee or by the University of Auckland Animal Ethics Committee.

All animals were accommodated to human contact and temporary restraint by daily lifting, moving, holding, and placement in the treadmill enclosure for 3 days before any exercise or surgical procedure. Each exercise bout consisted of 30 min of declined treadmill running (−16° grade, 15 m/min). Previous studies have shown that a single bout of exercise of this nature will induce focal muscle fiber injury in the soleus (Sol) and gastrocnemius and deep portions of the quadriceps femoris muscles, whereas the ankle plantar flexors remain unaffected (2, 18, 33).

Experiment 1: In vivo labeling of proliferating satellite cells. Eight groups of rats were used to examine the effects of acute and daily exercise on satellite cell proliferation in the Sol muscle. Rats were killed either as nonexercised (0Ex) controls (n = 6); 1 (n = 10); 2, 4, or 7 (n = 5/group) days after a single exercise bout (1Ex); or after two, four, or seven daily exercise bouts (DailyEx; n = 5/group) that were each of the same intensity and duration as 1Ex. Rats in the DailyEx groups were killed 24 h after the final bout of exercise. This ensured that the two-, four-, and seven-bout DailyEx were comparable to 1Ex groups with 2, 4, and 7 days of recovery, with regard to the time elapsed after the initial exercise bout.

A single injection of 5-bromo-2′-deoxyuridine (BrdU; Sigma Chemical, St. Louis, MO) in 0.9% sterile saline (50 mg/kg, 10 mg/ml ip) was administered 1 h before anesthetic and surgical procedures. Proliferating cell nuclei, including satellite cells, in the S phase of the cell cycle during this interval would incorporate the BrdU during DNA replication. Nonreplicating cell nuclei, including the postmitotic mature myofiber nuclei and quiescent satellite cells, would remain unlabeled.

Experiment 2: In vivo cumulative labeling of newly produced myonuclei. Continuous infusion of BrdU was used for the identification of myonuclei produced in the Sol, vastus intermedius (VI), and tibialis anterior (TA) muscles over a 7-day period. Miniosmotic pumps (ALZET 2ML1, Alza Scientific) filled with 2 ml of BrdU solution (25 mg/ml) were implanted in 0Ex rats (n = 6) and in rats directly after one exercise bout (1Ex; n = 6) or after the first of seven, once-daily exercise bouts (DailyEx; n = 6). Rats were first anesthetized with methoxyfluorane, and, under aseptic conditions, a preprimed (4 h in sterile saline at 37°C) pump was placed subcutaneously via a small incision made in the skin above the scapulae. The pumps, designed to deliver 10 μl solution/h over the implantation period, were left in place until after the death of the animals 7 days later. The volume of the remaining contents of each pump was then aspirated and measured to confirm adequate delivery of the solution (all contained ≤0.35 ml). The concentration of BrdU administered resulted in a circulating level of the chemical that far exceeds that of the endogenous thymidine (9), and thus all replicating satellite cell nuclei, and daughter nuclei that subsequently fused to become mature, postmitotic myonuclei, should have been labeled with BrdU during the exposure period. Despite some caution regarding the effects of BrdU on nuclear activity (3, 36), this same concentration of BrdU administered for 1–2 wk does not appear to inhibit in vivo satellite cell proliferation or fusion (6, 20, 29).

Tissue collection and processing. At death, rats were deeply anesthetized by an intraperitoneal injection of pentobarbital sodium (Somnotol, MTC Pharmaceuticals), and the Sol, VI, and TA muscles were excised. Animals were maintained under deep anesthesia until all procedures were completed and were then killed by anesthetic overdose. In experiment 1, samples from consistent midmuscle regions were coated in embedding medium (Tissue-Tek OCT, Miles, Naperville, IL), immersed in isopentane cooled by liquid nitrogen, and stored at −80°C until use. Transverse sections (10 μm thick) were later cut from the stored samples at −20°C, mounted serially onto adherent-coated slides, and stained for the identification of proliferating cell nuclei, nonproliferating myonuclei, fiber type, and general morphology. In experiment 2, muscle samples were fixed overnight (~18 h) in Carnoy’s solution and processed to obtain individual fiber segments free of interstitial cells and adherent fibroblasts with the use of procedures described previously (19, 28, 30). Muscle samples (n = 6 Sol, 3 TA, 3 VI per group) were rehydrated, washed in 0.1 M PBS, and incubated in collagenase (141 U/mg, 2.5 mg/ml PBS; type 3, Worthington Biochemical Lakewood, NJ) for 18 h at 37°C, with intermittent agitation. The individual fibers were then washed and stained for newly produced BrdU-labeled, and mature non-BrdU labeled, myonuclei.

Immunocyto- and histochemistry. A commercially available (Boehringer Mannheim Biochemical; Roche Diagnostics) mouse monoclonal IgG1 primary antibody directed against BrdU was used to detect the nuclei of cells that had incorporated the BrdU into their DNA in both muscle sections and individual fiber segments. Muscle samples were incubated sequentially with 0.03% hydrogen peroxide in PBS for 30 min, 10% normal horse serum (NHS) in PBS for 20 min, and the primary antibody, diluted 1:10 in the antibody kit-provided Tris buffer solution, for 35 min at 37°C. After reimmubination with NHS, samples were incubated with biotinylated anti-mouse IgG (Vector Laboratories, Burlingame, CA), diluted in 2% NHS in PBS for 35 min and then avidin-biotin peroxidase (Vectastain ABC Kit; Vector) for 30 min, and developed with an aminoethylcarbazole (30 min) or diaminobenzidine-nickel (5 min) substrate solution (Vector). Fibers and sections were counterstained with Mayer’s hematoxylin for the identification of non-BrdU-labeled nuclei. Technical controls were processed by using the replacement of each primary and/or its secondary antibody with the diluent only.

One set of BrdU-labeled sections was sequentially labeled for the identification of the fiber basal laminae by using a rabbit polyclonal anti-laminin (1:100; Dako) and a biotinylated, anti-rabbit secondary antibody (1:500; Amersham), followed by the avidin-biotin method as above but with a contrasting color substrate.

Sections from Sol after 2 days of DailyEx (n = 5) were also labeled for BrdU and/or the myogenic regulatory factor MyoD
(MoAb 5.8A, 1:40; BD Biosciences, San Jose, CA). These sections were intended to assess the number of BrdU-labeled nuclei situated within the periphery of muscle fibers that could be confirmed to be of myogenic origin. The muscle samples were selected based on the time since the initial exercise bout, when it was expected that they would exhibit a larger proportion of BrdU-labeled nuclei of nonmuscle origin, such as inflammatory or phagocytic cells, than nonexercised muscles, or after a longer recovery period (2, 33).

Sets of sections were also processed for slow (type I) and fast (type IIA, IIB, IId/x) adult myosin heavy chain (MHC) isoforms with the use of commercially available monoclonal mouse IgG1 antibodies (Novocastra Laboratories, Newcastle upon Tyne, UK) with standard indirect immunohistochemical methods, as previously described (34). Last, one set of sections was stained with routine hematoxylin and eosin for the identification of fibers with degenerative or regenerative characteristics indicative of prior injury (33). These characteristics included the infiltration by inflammatory or phagocytic cells, a disrupted cytoplasm, an atrophic and sharply angular appearance, an apparent “splitting,” a very small size and extrafascicular location, and/or the presence of internalized or centrally located myonuclei (33).

Image analysis and quantification. Muscle samples were examined by using one of two image analysis systems, each consisting of a light photomicroscope (Olympus America, Lake Success, NY; Nikon), color charge-coupled device camera (COHU, San Diego, CA; SPOT, Diagnostic Instruments, Sterling Heights, MI), personal computer, and image processing software (Mocha Image Analysis Software, Jandel Scientific, San Rafael, CA; ImagePro Plus, Media Cybernetics, Silver Spring, MD). Pixel-to-real-size conversions for each magnification were made by using images of a stage slide micrometer with 0.01-mm divisions.

In experiment 1, the number of BrdU-labeled nuclei within fibers was counted from entire sections of the Sol muscle. Criteria for the inclusion of BrdU-labeled nuclei as proliferating satellite cells included a peripheral location within and abutting the cell boundary and a distinct staining intensity and size. Later analyses showed no significant difference between the number of MyoD-positive nuclei per muscle section and the number of proliferating satellite cells as determined by the above criteria (P = 0.24). Double-labeling of sections with BrdU and MyoD using the current methods proved difficult because of the nuclear colocalization of both antigens and the particular difficulty of the detection of MyoD. Thus, although some of the sublaminal BrdU-labeled nuclei could be confirmed to be of myofiber origin, the possibility that some of these nuclei were of other cell types cannot be entirely excluded.

The number of fibers containing BrdU-labeled nuclei and the number of BrdU-labeled nuclei and hematoxylin-stained nuclei within each fiber were also tabulated from image fields (total area = 2.8 mm²) of each muscle section [582 ± 78 (SD) total fibers]. Satellite cell proliferation was described by the number of fibers containing BrdU-labeled nuclei and the number of BrdU-labeled nuclei calculated as a percentage of the number of total myonuclei (BrdU-labeled and non-BrdU-labeled nuclei within fibers). Individual fibers with BrdU-labeled nuclei were also type classified from fast MHC-stained serial sections. The percentage of fibers with degenerative or regenerative characteristics indicative of prior injury (33) was determined from hematoxylin-and-eosin-stained sections (627 ± 100 total fibers) of each Sol muscle. The overall fiber-type composition of the Sol, VI, and TA was determined from images (397 ± 114 fibers/muscle) of the muscles of the 0Ex rats (n = 6).

In experiment 2, mean fiber segment diameter and the number of BrdU-labeled and non-BrdU-labeled myonuclei were determined from images of one focal plane of 16 (Sol and VI) or 20 (TA) individual fiber segments from each identity-coded muscle. The fiber segments selected for analysis were intact, free of surface cells and connective tissue, and presented an undistorted view for imaging. A minimum of 1,000 myonuclei of each TA (1,030 ± 47) and 1,400 myonuclei of each Sol (2,474 ± 512) and VI (1,825 ± 371) muscle was tabulated.

Statistical analyses. One-way ANOVA or, where data did not follow a normal distribution, nonparametric analyses (Kruskal-Wallis) were used to compare satellite cell proliferation over time after 1Ex or DailyEx. A t-test was then used for the comparison of proliferation between 1Ex and DailyEx at each time point. One-way ANOVAs were also used to compare the percentage of newly produced myonuclei, total myonuclear number, and mean fiber diameter for each muscle among the different exercise groups (0Ex, 1Ex, DailyEx) or among TA, Sol, and VI under each exercise condition. For all tests, statistical significance was established at P = 0.05.

RESULTS

All rats examined over the entire 7-day period in the two experiments increased in body mass (1.7 ± 2.4%, P = 0.001, n = 28). However, there was no significant difference (P = 0.4) between the weight gain of 0Ex rats (2.6 ± 2.6%) and that of all rats exercised either once (1.8 ± 2.7%) or seven times (1.0 ± 1.6%). In experiment 2, Sol wet mass relative to body mass did not differ among 0Ex (0.51 ± 0.05 mg/g), 1Ex (0.48 ± 0.05 mg/g), and DailyEx (0.50 ± 0.05 mg/g) groups.

Experiment 1: Satellite cell proliferation. The number of BrdU-labeled proliferating satellite cell nuclei (Fig. 1) observed in whole Sol muscle sections was not different between 0Ex and any of the time points after 1Ex. However, in DailyEx rats, there was a significant increase in the number of proliferating nuclei after 2 days of exercise (Fig. 2).

The percentage of fibers with proliferating nuclei, calculated from image fields in muscle sections, was 0.4 ± 0.2% (1.3 ± 0.7 per 1,000 myonuclei) in 0Ex. A wide range in the incidence of such fibers (P = 0.36) was observed 1 day after 1Ex (0.8 ± 0.9%, 2.8 ± 3.2 per 1,000 myonuclei), with a consistent, low level of cell replication (0.1–0.4%, 0.8–1.4 per 1,000 myonuclei) observed 2, 4, and 7 days after 1Ex. The percentage of fibers with proliferating nuclei was, however, significantly greater in DailyEx than 0Ex rats (P = 0.04), being highest after two bouts of exercise (1.0 ± 0.2%, 3.4 ± 0.7 per 1,000 myonuclei).

The percentage of fibers demonstrating histological characteristics of prior injury was significantly greater 7 days after 1Ex (7.4 ± 1.7%) than in 0Ex (4.2 ± 1.7%, P = 0.03) or after DailyEx for 7 days (4.7 ± 0.8%, P = 0.01). A 5.9 ± 2.9% (P = 0.25) incidence of fibers with degenerative and regenerative characteristics was found 4 days after 1Ex, yet all other groups showed consistent mean levels (4.6–5.1%) of affected fibers.

Of the total number of fibers with BrdU-labeled nuclei, 12.4% reacted positively with the anti-fast MHC antibody. The overall fiber-type composition of
the Sol was 78% slow MHC, 16% fast MHC, and 6% slow and fast MHC reactive. Fiber-type composition of the VI was 42% slow MHC, 55% fast MHC, and 3% slow and fast; the TA was 5% slow MHC with the balance reacting for fast MHC.

Experiment 2: Myonuclear accretion. The continuous infusion of BrdU provided a cumulative, permanent identification of dividing satellite cells and their progeny, allowing the determination of newly produced myonuclei accrued within the muscle (Fig. 3).

The number of newly produced nuclei observed after the 7-day labeling period, expressed as a percentage of all myonuclei, for the TA, Sol, and VI of 0Ex, 1Ex, and DailyEx rats, is shown in Fig. 4. In the Sol and VI, DailyEx resulted in a greater percentage of new nuclei than did 1Ex and over double the percentage of new nuclei produced in 0Ex over the same 7-day period (all \( P < 0.05 \)). Exercise had no significant effect on the accretion of new myonuclei in the TA (\( P = 0.09 \)). The total number of myonuclei counted per millimeter of fiber segment length did not differ among 0Ex, 1Ex, and DailyEx groups for any of the muscles examined (Fig. 5). However, there were more (\( P < 0.05 \)) myonuclei per millimeter in Sol (126 ± 21) than in VI (96 ± 15) and more in Sol and VI than in TA (43 ± 2).

Mean muscle fiber segment diameters were not significantly different among 0Ex, 1Ex, and DailyEx groups for any muscle (Fig. 6).

DISCUSSION

Experiment 1: Satellite cell proliferation. The increased satellite cell proliferation seen in the Sol of rats that exercised daily for 2 days, compared with 0Ex or 1Ex, falls within the range calculated from prior data based on transverse sections of the muscle 12 h to 7 days after 2 h of level treadmill exercise (0.3 to 1.8% of fibers; Ref. 15), yet is lower than that observed 1 day after 90 min of downhill exercise (8). The exercise in the present study was shorter in duration and metabolically less demanding than that used in these prior studies, yet it has previously been shown to elicit fiber injury and regeneration in the Sol muscle (33, 34). We, therefore, anticipated that it would be sufficient to increase satellite cell proliferation, even after a single bout. However, the exercise only appeared to induce significant increases in fiber degenerative and regenerative characteristics indicative of prior injury at 7 days after 1Ex. Earlier or additional structural injury and sequelae not visible at the light microscopic level may have occurred and provided the stimulus for the increased activation and proliferation of satellite cells seen in the DailyEx rats. Alternatively, the proliferation of satellite cells may have been affected by stimuli associated with the exercise but independent of the collective histological indexes of injury of the muscle.
The timing of the increased satellite cell proliferation after the initial exercise bout meets the minimum duration required for fusion of satellite cells in growing rats (22, 32) and corresponds to the first detectable increases in replicating satellite cells after focal fiber injury (8, 31). The elevation of proliferation seen in rats subjected to a second day of exercise indicates that new satellite cells were recruited, because any cells already activated after the first bout of exercise would not yet have completed a full cell cycle (duration $\approx 32$ h; Ref. 29). The greater proliferation seen after two vs. one bout of exercise also implicates the second bout as an additional stimulus for the recruitment of available precursor cells. The subsequent decline in proliferation suggests that, thereafter, the exercise provided no further stimulus for the activation or replication of satellite cells, and/or that the responsiveness of satellite cells to the exercise stimulus was reduced.

It is apparent from the low level of proliferation in the 0Ex animals and at 2, 4, and 7 days after 1Ex that, in the adult rat Sol, some satellite cells are within the replicative cell cycle, even in the absence of an externally applied stimulus such as exercise. Thus an increased proliferation may rely on the recruitment of quiescent satellite cells (8, 15) and be related specifically to the exercise stimulus and/or the release of growth factors within the muscle. In this study, the
second daily bout of exercise may have provided the appropriate stimuli to maintain the entry and progression of these satellite cells through their replicative cycle.

The proliferation of satellite cells and morphological indexes of ongoing muscle-fiber degeneration and regeneration in the Sol muscle of 0Ex rats seen here and in prior studies (8, 15, 33) support the belief that satellite cells are recruited at low levels to contribute to a continual, possibly age-related (21), fiber turnover in this postural, high-use muscle.

The relative incidence of fibers containing proliferating satellite cell nuclei and expressing fast MHC compared with the overall percentage of such fibers in the Sol muscle is consistent with a lower availability and/or activation of satellite cells in these fiber types (12, 17). However, the data demonstrate that satellite cells in fibers expressing fast MHC within the predominantly slow Sol muscle proliferate in response to the use of the muscle during unaccustomed locomotory exercise. These fibers are recruited (11) and are susceptible to injury (33) during downhill exercise yet may not have the same regenerative capacity as the slow fibers.

Experiment 2: Myonuclear accretion. In the Sol and VI, daily exercise enhanced the accretion of new myonuclei compared with normal caged recovery after the initial bout of unaccustomed exercise.

The greater proportion of new myonuclei found after DailyEx compared with the 0Ex and 1Ex groups we accept as resulting from a correspondingly greater increase in the production of myonuclei. Although a concurrent loss of myonuclei by apoptotic or necrotic myo-cellular or myonuclear elimination may have occurred, this could not have accounted for the observed relative difference in the percentage of new nuclei between 0Ex and DailyEx muscles. The greater than twofold increase in new nuclei seen in Sol and VI with DailyEx would have required an extreme loss of myonuclei (e.g., to less than one-half in the DailyEx rats) that would have been detected by our myonuclear counts; these showed no significant change in any muscle over the 7-day period.

Myonuclear production is a function of the number of satellite cells dividing and the number of divisions of each particular cell. The latter is dependent on the duration of the cell cycle and the maintenance of each cell in an active replicative state. Hence, the newly produced myonuclei resulted either from the activation of a corresponding percentage of satellite cells each replicating once or from a smaller proportion of the available mitotic cells (and their progeny) completing more than one replicative cycle.

How could the continued exercise contribute to an enhanced accretion of new nuclei? The initiation, progression, and continuation (or termination) of myogenic cell proliferation are controlled by a number of positive and negative regulatory factors (13). The daily exercise may have increased blood or lymphatic flow and/or altered the release or distribution of such factors to and within the working muscles. The nature of the exercise and the extent of the injury incurred are also important to the regeneration of the tissue. In this study, the exercise was of moderate intensity and duration; involved submaximal, asynchronous, and graded recruitment of motor units within muscles; and could be tolerated by the rats on a daily basis. Based on the moderate metabolic demands and duration of the exercise, we neither expected nor intended to induce either an endurance training effect (i.e., increases in oxidative capacity) or hypertrophy of the muscle fibers. The fiber injury and regeneration after the exercise were focal in nature, segmental along fibers, and moderate in extent (e.g., 7.5% of fibers in Sol). The recovery of the muscle would not have been compromised by a disruption of the blood supply or innervation or the connective tissue scarring that can occur with severe injury. In addition, data from experiment 1 and our previous study (33) showed that morphological indexes of prior injury in affected muscles were less extensive after daily exercise than at the same time point after a single bout of exercise with only normal caged activity on subsequent days. Hence, in the present study, some fiber injury in Sol and VI probably resulted from the first bout of exercise, and the myonuclei produced thereafter were contributing to the repair, regeneration, or regrowth of fibers in the exercised muscles. The continued functional overloading of the muscles may have enhanced or accelerated these processes. The lack of change in mean fiber diameter and wet mass of the muscles with 7 days of exercise or passive recovery supports the idea that the accretion of new myonuclei was related to fiber injury and repair, rather than mature fiber hypertrophy, during this time. However, the influence of newly produced nuclei on subsequent fiber adaptation with continued exercise training is not known.

The observed degree of satellite cell proliferation and accretion in each of the different leg muscles may depend on the extent of the initial injury and the nature of the loading subsequently imposed. Motor units of the Sol are heavily recruited during locomotory activities (14), and the muscle is susceptible to treadmill exercise-induced injury (2, 33) and has a higher number of satellite cells (e.g., 5–9% of myofiber nuclei) than less oxidative muscles (12). Hence, the greater accretion of new myofiber nuclei after exercise in this muscle may be a function of these characteristics. The accretion of new nuclei in VI to the same extent as in the Sol was not expected, based on the faster fiber-type composition of the muscle and the probable lower availability of satellite cells (e.g., 3–4%; Ref. 37). However, the muscle would be well recruited and can be injured during treadmill exercise (18). In contrast, the TA is most active during non-weight-bearing phases of the rat’s gait, comprises predominantly fast-twitch motor units that are infrequently recruited during normal activities, and incurs little injury from downhill treadmill exercise (33).
The cumulative satellite cell mitotic activity in injured or exercised adult rat muscles has not, to our knowledge, been reported previously. However, the number of myofiber nuclei accrued in all muscles over the 7-day period was lower than that found recently in the Sol (14%; Ref. 23) and plantaris (11%; Ref. 7) of nonexercised, young adult rats after 14 days of BrdU infusion. This difference was likely a function of our shorter infusion period (the delivery rates of the BrdU from the pumps were the same) and perhaps the different strain and sex of the rats. However, because our labeling after 7 days was less than one-half of that previously reported, the early dividing satellite cells would have had to remain mitotically active, going through more than one replicative cycle before fusion, to accelerate myonuclear production over the 14-day period. The consistent proliferation indexes we observed in experiment I do not support this possibility, and this discrepancy remains to be explained.

The use of intact, isolated fibers may result in an underestimate of the number of new myonuclei by the exclusion of those associated with very small or necrotic fibers. However, the exercise was sufficient to enhance myonuclear accretion even in the mature, intact fibers that were examined over the 7-day period.

Our findings suggest that daily exercise evokes further satellite cell cycle entry and/or maintenance of proliferating cells within the cell cycle above that after the initial bout of exercise alone. In addition, over a 7-day period, daily exercise results in a greater net accretion of newly produced myonuclei in the Sol and VI muscles compared with that after a single bout of exercise with a subsequent passive recovery. The increased proportion of new myonuclei may be due to their greater production to augment the available transcriptional regulation of proteins required for fiber repair, regeneration, or regrowth during this time. The implications of greater new myonuclear accrual on satellite cell availability and fiber adaptability with continued exercise training have yet to be determined.

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REFERENCES