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Morphology and Anabolic Response of Skeletal Muscles Subjected to Eccentrically or Concentrically Biased Exercise

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Context: Long-term eccentric exercise is known to promote muscle growth better than concentric exercise, but its acute effect on muscle is not well understood because of misinterpreted modeling and in situ and in vitro stretch protocols. Knowing if the initial bout of eccentric exercise promotes muscle growth and limits damage is critical to understanding the effect of this mode of exercise.

Objective: To directly evaluate the immediate effects of eccentric and concentric exercises on untrained muscle when fiber strains were physiological and exercise doses were comparable.

Design: Controlled laboratory study.

Setting: Laboratory.

Patients or Other Participants: A total of 40 skeletally mature male Long-Evans rats (age = 16 weeks, mass = 452.1 ± 35.2 g) were randomly assigned to an eccentric exercise (downhill walking, n = 16), concentric exercise (uphill walking, n = 16), or control (no exercise, n = 8) group.

Intervention(s): Rats were exposed to a single 15-minute bout of eccentric or concentric exercise on a motorized treadmill and then were euthanized at 6 or 24 hours postexercise. We harvested the vastus lateralis muscle bilaterally.

Main Outcome Measure(s): The percentage increase or decrease in protein abundance in exercised animals relative to that in unexercised control animals was evaluated as elevated phosphorylated $p70^{S6k}$ relative to total $p70^{S6k}$. Fiber damage was quantified using immunoglobulin G permeability staining. One-way analysis of variance and post hoc Tukey tests were performed.

Results: Rats exposed to eccentric exercise and euthanized at 24 hours had higher percentage response protein synthesis rates than rats exposed to eccentric exercise and euthanized at 6 hours ($P = .02$) or to concentric exercise and euthanized at 6 ($P = .03$) or 24 ($P = .03$) hours. We assessed 9446 fibers for damage and found only 1 fiber was infiltrated (in the concentric exercise group euthanized at 6 hours). Furthermore, no between-groups differences in immunoglobulin G fluorescent intensity were detected ($P = .94$).

Conclusions: Incorporating eccentric exercise is a simple, universally available therapeutic intervention for promoting muscle recovery. A single 15-minute dose of eccentric exercise to a novice muscle can better exert an anabolic effect than a comparable dose of concentric exercise, with very limited evidence of fiber damage.

Key Words: lengthening contractions, mammalian target of rapamycin pathway, muscle injury

Key Points

- A single 15-minute dose of eccentric exercise to an untrained muscle better exerted an anabolic effect on muscle than a comparable dose of concentric exercise without damaging muscle fibers.
- The potential for the clinical application of eccentric exercise is high for patients needing to add or restore muscle tissue.

Restoring muscle strength after joint injury is fundamental to preventing disability and biomechanical alterations that increase the risk of chronic joint disease.[1,2] Increased muscle strength is known to be advantageous to joint health and quality of life, and, contrary to current bias, it also appears that loading the muscle during lengthening contractions (eccentric exercise) is critical to promoting unique adaptations in muscle that are associated with the recovery of function.[3] Due to misapplied mathematic modeling[4,5] and in vitro stretch protocols,[6–8] the notion that eccentric exercise leads to injury has been ingrained in the literature.[8,9] In response, clinicians have relied on concentric exercise postinjury to improve the recovery of muscle function. Unfortunately, concentric exercise does not adequately restore muscle strength.[10,11] Eccentric exercise differs from traditional concentric training in that it develops muscle tension to control motion. This ability to mechanically engage muscle

is the key to initiating stress-sensing molecular events that promote muscle growth.[12] Hence, it seems reasonable that incorporating eccentric exercise early in rehabilitation is a logical and effective intervention to promote muscle recovery.

Although long-term eccentric exercise (6–8 weeks) is beneficial to muscle,[3,13,14] the acute ability of this exercise to promote muscle growth and limit fiber damage is not well understood. This is because investigators have forced excessive exercise doses using exogenous stimulation parameters with hundreds of repetitions[7] and large, non-physiological fiber strains meant to cause fiber[15,16] and myotendinous junction injury.[17] Knowing if the initial bout of eccentric exercise causes severe muscle damage under normal physiological conditions is critical to fully understanding the safety of eccentric exercise and encouraging patient compliance during therapeutic exercise. Comparing the anabolic response in muscle after comparable doses of exercise is essential to demonstrating the therapeutic potential of eccentric exercise and encouraging its adoption and use early in rehabilitation protocols. Therefore, the purpose of our study was to directly evaluate the immediate effects of eccentric and concentric exercises on untrained muscle when fiber strains were physiological and exercise doses were comparable. Given that skeletal muscle structure and its molecular mechanisms governing function and malleability are highly conserved across species,[18,19] we used an in vivo rat[20] model of constrained ambulation to better control the exercise dose. Downhill or uphill walking was used to subject the quadriceps muscles of the rodents to eccentric or concentric exercise, respectively. This protocol enabled investigators to examine the effect of a single bout of eccentric exercise on (1) muscle protein synthesis and (2) fiber injury compared with concentric exercise using a translational rodent model of exercise.

METHODS

Participants

We obtained and studied 40 healthy adult male (age = 16 weeks, mass = 452.1 ± 35.2 g) Long-Evans rats (Envigo Laboratories, Indianapolis, IN) with the approval of the Institutional Animal Care and Use Committee at the University of Connecticut. All animals were housed in individual cages within the vivarium and allowed to consume food and water ad libitum for the duration of the study.

Exercise Interventions

We used a randomized controlled laboratory study design. Experiments were conducted using a constrained rodent model, and rats were randomly assigned to an eccentric exercise (downhill walking, n = 16), concentric exercise (uphill walking, n = 16), or control (no exercise, n = 8) group. After a 1-week laboratory acclimatization period, rats began their respective exercise protocol based on group randomization. Rats in the exercise groups walked on a motor-driven treadmill (model EXER 3/6 treadmill; Columbus Instruments, Columbus, OH) at 16 m/min on a decline of 16° (eccentric exercise group) or on an incline of 16° (concentric exercise group).[20] The exercise comprised three 5-minute bouts of continuous walking with 2 minutes of rest between bouts, for a total treadmill time of 19 minutes. After the exercise, the rats were returned to their individual cages. They were euthanized at 6 or 24 hours postexercise (n = 8 allocated to each time), consistent with reports that these postexercise times are the peak hours of protein synthesis and fiber damage.[21–23] The vastus lateralis (VL) muscle was harvested bilaterally and immediately flash frozen in liquid nitrogen, stored in dry ice, and subsequently stored at −80°C. One VL muscle from each animal was then used for protein-synthesis analysis, and the other VL muscle was used for fiber-damage analysis.

Protein Synthesis

We used traditional Western blot procedures[24] to detect activation of the mammalian target of rapamycin (mTOR) pathway via the downstream target of phosphorylated $p70^{S6k}$. This measure of protein synthesis, elevated phosphorylated $p70^{S6k}$ relative to total $p70^{S6k}$, was selected because it is associated with greater muscle growth in both animals[21,25,26] and humans.[27,28] The central third of the VL muscle tissue (approximately 20 mg) was sectioned and homogenized in a radioimmunoprecipitation assay buffer (product R0278; Sigma-Aldrich, Inc, St Louis, MO) with a Halt Protease and Phosphatase Inhibitor Single-Use Cocktail (100X; Thermo Fisher Scientific, Waltham, MA). After homogenization, the samples were centrifuged at 5000 rpm at 4°C for 5 minutes. The supernatant was removed, and total protein concentration (in mg/mL) was determined using a bicinchoninic acid protein assay kit (Pierce BCA Protein Assay Kit; Thermo Fisher Scientific). Next, 30 μg of the supernatant was calculated for each sample, diluted 1 : 1 with 450 μL of a 2× Laemmli sample buffer and 50 μL of a 1-M solution of dithiothreitol (products 161-0737 and 1610610, respectively; Bio-Rad Laboratories, Inc, Hercules, CA), and boiled with a heating block at approximately 95°C for 5 minutes. Samples were loaded into precast gels (10% Criterion Tris-HCL Protein Gel, 18-well, 30 μL; Bio-Rad Laboratories, Inc) alongside a protein ladder (500 μL Precision Plus Protein Dual Color Standards; Bio-Rad Laboratories, Inc) and were electrophoretically resolved and subsequently transferred to a polyvinylidene difluoride membrane activated via 100% methanol membrane (product 162-0177; Bio-Rad Laboratories, Inc). After transfer, the membrane was incubated in Ponceau S solution (Sigma-Aldrich, Inc) for visualization of the protein and assurance of equal loading in all lanes. Membranes were washed with tris-buffered saline and 0.1% Tween 20 (products BP2471500 and BP337100, respectively; Thermo Fisher Scientific) and subsequently incubated for 1 hour at room temperature in a blocking buffer (tris-buffered saline and Odyssey Blocking Buffer [product 927; LI-COR Inc, Lincoln, NE]). After 1 hour, the blocking solution was poured off, and the membranes were incubated overnight at 4°C in the primary antibodies in 1 : 1000 dilutions of phosphorylated and total p70S6k (antibodies #9206 and #9202, respectively; Cell Signaling Technology, Inc, Danvers, MA). The membranes were subsequently washed and incubated at room temperature for 1 hour in secondary antibodies in 1 : 10 000 dilutions of phosphorylated $p70^{S6k}$ (antibody #A32729; Goat Anti-Mouse IgG [H + L], Superclonal Recombinant Secondary Antibody, Alexa Fluor 680; Thermo Fisher Scientific) and total $p70^{S6k}$ (antibody #A32735; Goat Anti-Rabbit IgG [H + L] Highly

Cross-Absorbed Secondary Antibody, Alexa Fluor Plus 800; Thermo Fisher Scientific).

The levels of phosphorylated and total $p70^{S6k}$ were quantified using an Odyssey infrared imaging system (LI-COR Inc) and Image Studio software (LI-COR Inc). After the background was subtracted, the captured intensities of the phosphorylated and total $p70^{S6k}$ bands were individually quantified. To minimize the effects arising from multiple blots and improve data reliability, we calculated an in-lane total $p70^{S6k}$ normalization factor for each blot. We accomplished this by identifying the highest total $p70^{S6k}$ signal on each blot and using this signal intensity to normalize all other total $p70^{S6k}$ signal intensities. The phosphorylated $p70^{S6k}$ signal was divided by the total $p70^{S6k}$ in-lane normalization factor to produce the normalized experimental signal for each lane. To determine the percentage increase or decrease in protein abundance relative to that of unexercised control animals, the percentage response equation was applied to each lane (Equation):

$$\text{Percentage Response} = \frac{(\text{Normalized Experimental Signal for Each Lane})}{(\text{Normalized Experimental Signal for a Single Control on the Blot} - 1)} \times 100.$$

Fiber Damage

Fiber damage was measured using positive myofibrillar staining. This technique assesses damage to the cellular membrane by quantifying muscle fibers that are lysed and permit the influx of the large extracellular immunoglobulin G (IgG) complex into the fiber. The IgG infiltration can be visualized using fluorescent dye.[29] In preparation for IgG staining, we sectioned frozen midbelly cross-sections of the VL (8 μm) and mounted them on glass slides. Slides were then fixed in ice-cold acetone (100%) for 10 minutes, washed 3 times with phosphate-buffered saline (PBS), and incubated in 5% bovine serum albumin solution in PBS for 20 minutes at room temperature. The primary antibody (Anti-Mouse IgG [whole molecule] FITC antibody, F0257; Sigma-Aldrich, Inc) was applied, and the slides were incubated in this solution overnight at 4°C. Next, we washed the slides twice in the 5% bovine serum albumin in PBS solution and once in PBS. Slides were then stained with 4′,6-diamidino-2-phenylindole (product D9542; Sigma-Aldrich, Inc) and coverslipped with VECTASHIELD Antifade Mounting Medium (product H-1000; Vector Laboratories, Burlingame, CA). Images were captured using a Zeiss Axio Imager M1 microscope (Carl Zeiss Microscopy GmbH, Göttingen, Germany). Five random fields of each muscle were photographed at a magnification of ×100 and subsequently analyzed using ZEN blue software (Carl Zeiss Microscopy GmbH). Fluorescent intensity was quantified by the densiometric mean of each fiber (in arbitrary units). A trained, blinded assessor (M.S.W.) counted the IgG infiltration, a marker of overt fiber damage.

Statistical Analysis

For the comparisons of exercise groups, 1-way analysis of variance was performed using GraphPad Prism statistical software (version 8.2.0; GraphPad Software, San Diego, CA). When we observed a difference, we applied a post hoc Tukey test to identify the differences between groups. Outliers that were 2 standard deviations from the group mean were removed from the analysis. All values are reported as mean ± standard error of the mean. We set the α level a priori at .05.

RESULTS

Protein Synthesis

Control group rats were used to provide normalized signaling of the phosphorylated $p70^{S6k}$ signal and total $p70^{S6k}$ relative to exercise groups (Equation). Therefore, this group was not included in the analysis of variance, as it was mathematically accounted for in the percentage response equation. A difference in VL muscle protein response was detected between exercise groups ($F_{3,54} = 4.50$, $P = .006$; Figure 1). The post hoc test revealed that rats in the eccentric exercise group euthanized at 24 hours postexercise had a higher VL muscle percentage response than rats in the eccentric exercise group euthanized at 6 hours postexercise ($P = .02$) and rats in the concentric exercise group euthanized at 6 ($P = .03$) or 24 ($P = .03$) hours postexercise. No other between-groups differences were observed.

Fiber Damage

We observationally quantified the fluorescent intensity of 9446 fibers via the densiometric mean and inspected them for damage using positive myofibrillar staining (Figure 2). Notably, only 1 fiber was infiltrated. This fiber was found in the concentric exercise group euthanized at 6 hours postexercise (Figure 2B). No between-groups differences in fluorescent intensity were detected ($F_{4,30} = 0.184$, $P = .94$).

DISCUSSION

The acute ability of eccentric exercise to promote muscle growth and limit muscle damage is not well understood. This misunderstanding is, in part, due to classic animal experiments in which investigators[6–8,15–17,22,30,31] isolated muscles in situ and single fibers in vitro and applied lengthening stretches of a very high magnitude to induce fiber injury, far exceeding the physiological strains experienced during in vivo muscle contractions. Other researchers have counteracted the concerns with these experiments by studying in vivo exercise protocols (eg, the muscle is kept intact and the system is not physiologically altered). Specifically, Armstrong et al[22] examined the acute effect of eccentric exercise on an untrained muscle by using downhill and uphill walking protocols in rats to eccentrically and concentrically strain the quadriceps muscle, respectively. However, the dose of exercise used in these experiments (90 minutes of activity) was specifically chosen to cause injury, limiting the clinical translation.[22] A similar experiment has been conducted in humans, with researchers[31] exposing novice men to eccentric exercise. However, again, the authors selected such high doses that muscle injury was unavoidable (30 minutes of continuous eccentric contractions to the untrained quadriceps muscle).[31] Remarkably, if the dose of eccentric exercise is

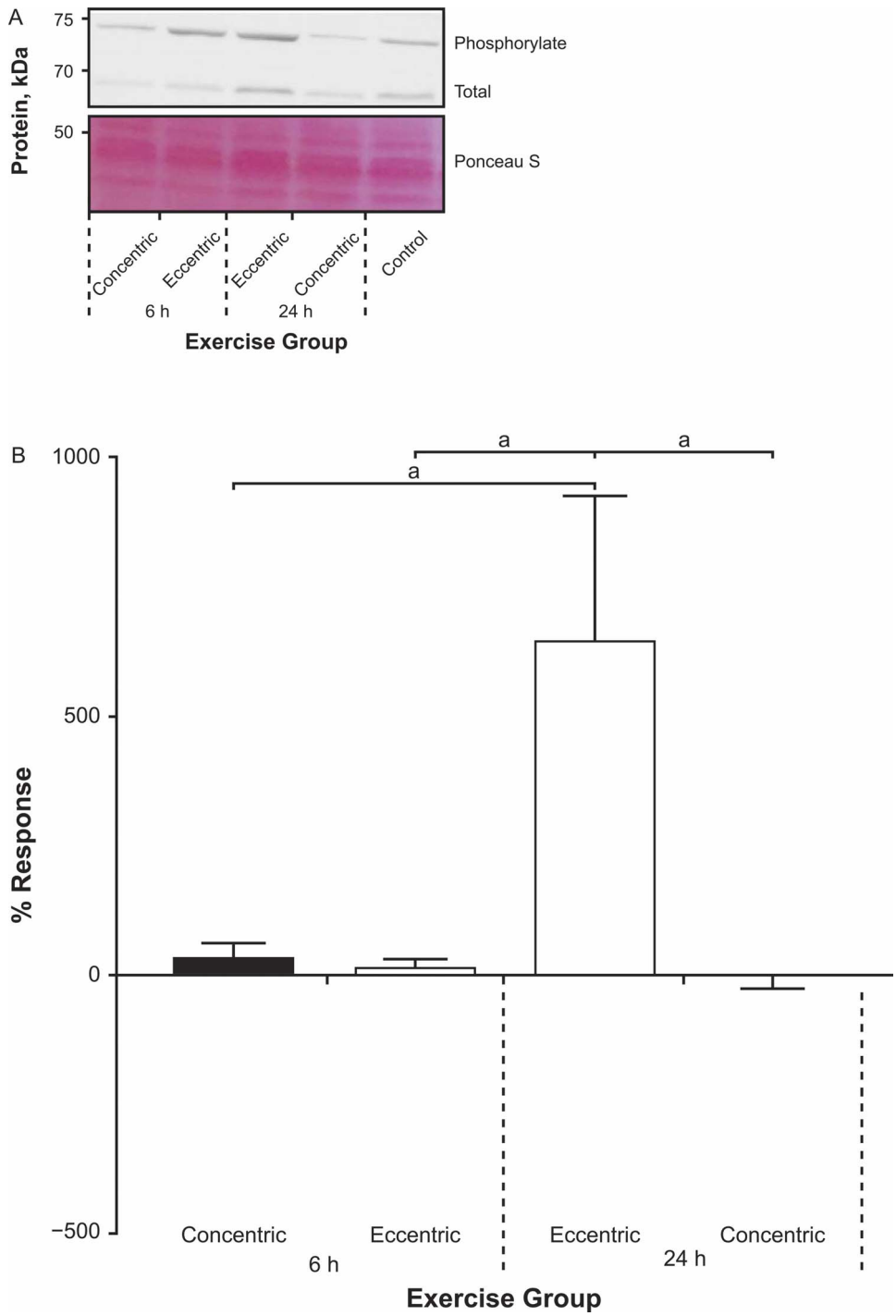

Figure 1. Effect of exercise on protein synthesis. A, To minimize the effects arising across multiple blots and improve data reliability, an in-lane total p70^{S6k} normalization factor was calculated for each blot. B, Calculated percentage response of the phosphorylated p70^{S6k} signal by the total p70^{S6k}. Data are mean ± standard error of the mean. [a] Different from the eccentric exercise group euthanized 24 hours postexercise ($P < .05$).

tempered, little is known about the acute response of the untrained muscle to eccentric exercise.

Our data demonstrated that a single 15-minute dose of eccentric exercise to an untrained muscle promoted a protein synthesis response and was not associated with muscle-fiber damage. We believe these data are critically important because knowing if an acute bout of eccentric exercise causes severe muscle damage that precedes long-term adaptations[3,13,14] is essential to encouraging patient compliance during the initial phases of exercise. These findings have great potential for musculoskeletal rehabilitation, as strength is not commonly restored after joint injury[1,2,10] and incorporating eccentric exercise to promote an anabolic response is a simple, universally available therapeutic intervention to promote muscle growth.

Distinctly different from the actions of muscle during concentric contractions, eccentric contractions mechanical-

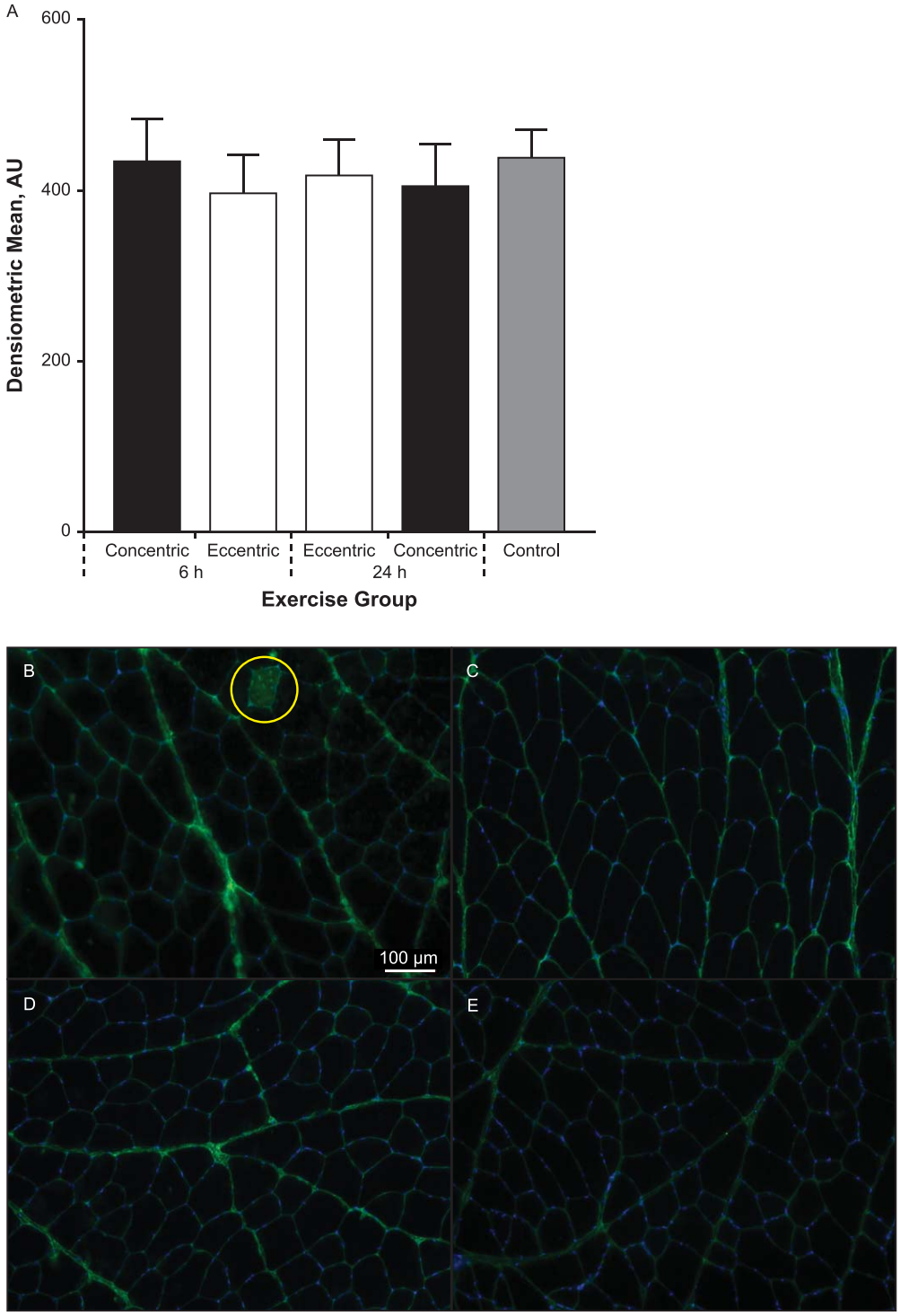

Figure 2. Effect of exercise on muscle-fiber damage. A, Calculated fluorescent intensity. Representative images of IgG staining (green) of the B, concentric, and C, eccentric, exercise groups euthanized at 6 hours postexercise and the D, eccentric, and E, concentric, exercise groups euthanized at 24 hours postexercise. B, The yellow circle surrounds a damaged (infiltrated) fiber.

ly tension the muscle in a unique way that triggers key molecular events that promote muscle growth.[12,32] Specifically, titin, the giant elastic protein that spans the entire length of the sarcomere, is involved in a mechanotransduction-signaling complex that regulates tissue growth.[12] Briefly, when the sarcomere is elongated, titin unfolds at the I-band. This unfolding allows for the phosphorylation of titin kinase.[12] Phosphorylation of titin kinase begins a cascade of events that ultimately promote muscle growth.[12] Importantly, only a positive strain of the sarcomere, achieved via eccentric contractions, triggers this pathway, and more force per active fiber increases the mechanical stimulus that, in turn, increases protein synthesis rates.[12,32] Over time, this unique mechanism will likely lead to the accumulation of new protein,[21,25,26] suggesting that the immediate incorporation of eccentric exercise in an

exercise regime is likely an effective intervention to promote muscle growth.

The mTOR pathway is arguably the most well-recognized pathway that leads to protein synthesis. Total $p70^{S6k}$ is a downstream target of the mTOR pathway and is associated with greater muscle growth in both animals[21,25,26] and humans.[27,28] Enhanced signaling can be detected by probing the muscle for evidence of phosphorylated relative to total $p70^{S6k}$ (eg, newly created protein relative to housekeeping levels of protein). Most data[21,27] have shown that a greater increase in muscle protein synthesis rates generally leads to long-term adaptations in muscle size and strength. However, some investigators[33,34] have shown that elevated $p70^{S6k}$ signaling does not always lead to muscle growth. In the case of overtraining or exercising to exhaustion, phosphorylated $p70^{S6k}$ levels can be increased, but oxidative stress is thought to inhibit growth,[33] ultimately leading to muscle loss.[34] Other researchers[35] have also reported that mTOR may be involved in the repair of muscle damage associated with eccentric exercise. Emerging contemporary data[36] have demonstrated that protein translation through eukaryotic translation initiation factor 4E–binding protein 1 activation, which is believed to be triggered by $p70^{S6k}$, is not always sufficient to promote translation. Given the limited exposure to continuous exercise in our experiments (three 5-minute bouts of exercise separated by 2 minutes of rest) and the sparse fiber damage observed, we contend that the acute activation of the mTOR pathway in our study will likely lead to muscle growth. Future experiments with protracted time-series data are needed to confirm or refute this hypothesis.

The time course of protein synthesis rates can vary. An animal experiment[21] showed that, after a single high-frequency electrical-stimulation protocol, acute activation of $p70^{S6k}$ peaks at 6 hours postexercise. Other researchers[37] observed that human muscle protein synthesis rates peaked at 24 hours after a single bout of biceps exercises. Given the variance in the muscle-protein synthesis rates in the literature, we probed 2 key time frames postexercise to detect a response. Our data demonstrated that protein synthesis peaked after 24 hours of a single bout of in vivo eccentric exercise. These results vary from those previously reported[21] in animal experiments. We believe this difference may be due to the experimental environment (in vivo versus in situ) and method of exercise (eccentric versus electrical stimulation).

Compared with many classic animal experiments in which researchers[6–8,22,30,31] studied eccentric exercise using in situ and in vitro exercise protocols, very limited fiber damage was observed in our experiment. We believe this was primarily due to the elastic properties of the intact muscle-tendon unit. Tendons act as a mechanical buffer that reduces impact to the muscle. Specific to eccentric contractions, tendons absorb energy during a brief rapid event (eg, landing from a jump) and then release this energy to the active muscle during lengthening contractions. This system is inherently built to protect muscles from damage. Another consideration is that the quadriceps muscles are highly pennate. During contraction, a change in muscle length is the net result of cross-bridging and the rotation of muscle fibers along the muscle's line of action, which leads to a change in muscle-pennation angle. An increased pennation angle (due to more fiber rotation) is a protective mechanism of the muscle and an indicator that the muscle-tendon unit has become decoupled from the muscle fascicle.[38] This decoupling leads to relatively minimal stretching of the fascicle, as the muscle-tendon unit absorbs most of the lengthening contractions.[38] In this way, pennate muscles are uniquely protected from fascicle strain and, thus, muscle-fiber damage. These data provide further evidence that muscles such as the quadriceps would uniquely benefit from eccentric exercise. However, a limitation of our work was that the time frames were selected to optimize capture of muscle protein synthesis rates. Evidence of muscle damage can occur up to 72 hours postexercise. Given the limited fiber damage observed at 24 hours, we anticipate that muscle damage present at later times would not be statistically different from that at earlier times. However, these data need to be confirmed with future experiments.

CONCLUSIONS

A single 15-minute dose of eccentric exercise to a novice muscle was better at exerting an anabolic effect on muscle than a comparable dose of concentric exercise. Furthermore, no muscle-fiber damage was observed. The possibilities for the clinical application of eccentric exercise are promising for patients seeking to build muscle tissue.

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REFERENCES

1. Andriacchi TP, Mundermann A, Smith RL, Alexander EJ, Dyrby CO, Koo S. A framework for the in vivo pathomechanics of osteoarthritis at the knee. *Ann Biomed Eng*. 2004;32(3):447–457.
2. Palmieri-Smith RM, Thomas AC. A neuromuscular mechanism of posttraumatic osteoarthritis associated with ACL injury. *Exerc Sport Sci Rev*. 2009;37(3):147–153.
3. Fridén J. Changes in human skeletal muscle induced by long-term eccentric exercise. *Cell Tissue Res*. 1984;236(2):365–372.
4. Hill AV. The mechanics of active muscle. *Proc R Soc Lond B Biol Sci*. 1953;141(902):104–117.
5. Morgan DL. New insights into the behavior of muscle during active lengthening. *Biophys J*. 1990;57(2):209–221.
6. Faulkner JA, Jones DA, Round JM. Injury to skeletal muscles of mice by forced lengthening during contractions. *Q J Exp Physiol*. 1989;74(5):661–670.
7. McCully KK, Faulkner JA. Injury to skeletal muscle fibers of mice following lengthening contractions. *J Appl Physiol (1985)*. 1985;59(1):119–126.
8. McCully KK, Faulkner JA. Characteristics of lengthening contractions associated with injury to skeletal muscle fibers. *J Appl Physiol (1985)*. 1986;61(1):293–299.
9. Proske U, Allen TJ. Damage to skeletal muscle from eccentric exercise. *Exerc Sport Sci Rev*. 2005;33(2):98–104.
10. Lepley LK. Deficits in quadriceps strength and patient-oriented outcomes at return to activity after ACL reconstruction: a review of the current literature. *Sports Health*. 2015;7(3):231–238.
11. Palmieri-Smith RM, Thomas AC, Wojtys EM. Maximizing quadriceps strength after ACL reconstruction. *Clin Sports Med*. 2008;27(3):405–424.
12. Puchner EM, Alexandrovich A, Kho AL, et al. Mechanoenzymatics of titin kinase. *Proc Natl Acad Sci U S A*. 2008;105(36):13385–13390.

13. Gerber JP, Marcus RL, Dibble LE, Greis PE, Burks RT, Lastayo PC. Safety, feasibility, and efficacy of negative work exercise via eccentric muscle activity following anterior cruciate ligament reconstruction. *J Orthop Sports Phys Ther*. 2007;37(1):10–18.
14. Lepley LK, Wojtys EM, Palmieri-Smith RM. Combination of eccentric exercise and neuromuscular electrical stimulation to improve quadriceps function post-ACL reconstruction. *Knee*. 2015;22(3):270–277.
15. Lieber RL, Woodburn TM, Friden J. Muscle damage induced by eccentric contractions of 25% strain. *J Appl Physiol (1985)*. 1991;70(6):2498–2507.
16. Brooks SV, Zerba E, Faulkner JA. Injury to muscle fibres after single stretches of passive and maximally stimulated muscles in mice. *J Physiol*. 1995;488(pt 2):459–469.
17. Äärimaa V, Rantanen J, Best T, Schultz E, Corr D, Kalimo H. Mild eccentric stretch injury in skeletal muscle causes transient effects on tensile load and cell proliferation. *Scand J Med Sci Sports*. 2004;14(6):367–372.
18. Egerman MA, Glass DJ. Signaling pathways controlling skeletal muscle mass. *Crit Rev Biochem Mol Biol*. 2014;49(1):59–68.
19. Lowe DA, Alway SE. Animal models for inducing muscle hypertrophy: are they relevant for clinical applications in humans? *J Orthop Sports Phys Ther*. 2002;32(2):36–43.
20. Butterfield TA, Leonard TR, Herzog W. Differential serial sarcomere number adaptations in knee extensor muscles of rats is contraction type dependent. *J Appl Physiol (1985)*. 2005;99(4):1352–1358.
21. Baar K, Esser K. Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. *Am J Physiol*. 1999;276(1):C120–C127.
22. Armstrong RB, Ogilvie RW, Schwane JA. Eccentric exercise-induced injury to rat skeletal muscle. *J Appl Physiol Respir Environ Exerc Physiol*. 1983;54(1):80–93.
23. Tidball JG, Villalta SA. Regulatory interactions between muscle and the immune system during muscle regeneration. *Am J Physiol Regul Integr Comp Physiol*. 2010;298(5):R1173–R1187.
24. White JR, Confides AL, Moore-Reed S, Hoch JM, Dupont-Versteegden EE. Regrowth after skeletal muscle atrophy is impaired in aged rats, despite similar responses in signaling pathways. *Exp Gerontol*. 2015;64:17–32.
25. Bodine SC. mTOR signaling and the molecular adaptation to resistance exercise. *Med Sci Sports Exerc*. 2006;38(11):1950–1957.
26. Nader GA, McLoughlin TJ, Esser KA. mTOR function in skeletal muscle hypertrophy: increased ribosomal RNA via cell cycle regulators. *Am J Physiol Cell Physiol*. 2005;289(6):C1457–C1465.
27. Terzis G, Georgiadis G, Stratakos G, et al. Resistance exercise-induced increase in muscle mass correlates with p70S6 kinase phosphorylation in human subjects. *Eur J Appl Physiol*. 2008;102(2):145–152.
28. Koopman R, Zorenc AH, Gransier RJ, Cameron-Smith D, van Loon LJ. Increase in S6K1 phosphorylation in human skeletal muscle following resistance exercise occurs mainly in type II muscle fibers. *Am J Physiol Endocrinol Metab*. 2006;290(6):E1245–E1252.
29. Zou K, Huntsman HD, Carmen Valero M, et al. Mesenchymal stem cells augment the adaptive response to eccentric exercise. *Med Sci Sports Exerc*. 2015;47(2):315–325.
30. Lieber RL, Fridén J. Mechanisms of muscle injury gleaned from animal models. *Am J Phys Med Rehabil*. 2002;81(suppl 11):S70–S79.
31. Friden J, Sjöström M, Ekblom B. Myofibrillar damage following intense eccentric exercise in man. *Int J Sports Med*. 1983;4(3):170–176.
32. Stahl SW, Puchner EM, Alexandrovich A, Gautel M, Gaub HE. A conditional gating mechanism assures the integrity of the molecular force-sensor titin kinase. *Biophys J*. 2011;101(8):1978–1986.
33. Takegaki J, Ogasawara R, Tamura Y, et al. Repeated bouts of resistance exercise with short recovery periods activates mTOR signaling, but not protein synthesis, in mouse skeletal muscle. *Physiol Rep*. 2017;5(22):e13515.
34. Goodman CA. The role of mTORC1 in mechanically-induced increases in translation and skeletal muscle mass. *J Appl Physiol (1985)*. 2019;127(2):581–590.
35. De Souza RWA, Aguiar AF, Carani FR, Campos GER, Padovani CR, Silva MDP. High-intensity resistance training with insufficient recovery time between bouts induce atrophy and alterations in myosin heavy chain content in rat skeletal muscle. *Anat Rec (Hoboken)*. 2011;294(8):1393–1400.
36. Baumann CW, Rogers RG, Otis JS, Ingalls CP. Recovery of strength is dependent on mTORC1 signaling after eccentric muscle injury. *Muscle Nerve*. 2016;54(5):914–924.
37. MacDougall JD, Gibala MJ, Tarnopolsky MA, MacDonald JR, Interisano SA, Yarasheski KE. The time course for elevated muscle protein synthesis following heavy resistance exercise. *Can J Appl Physiol*. 1995;20(4):480–486.
38. Azizi E, Roberts TJ. Geared up to stretch: pennate muscle behavior during active lengthening. *J Exp Biol*. 2014;217(pt 3):376–381.

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