Limited myogenic response to a single bout of weight-lifting exercise in old rats

TETSURO TAMAKI,1,2 SHUICHI UCHIYAMA,3 YOSHIYASU UCHIYAMA,4 AKIRA AKATSUKA,5 SHINICHI YOSHIMURA,6 ROLAND R. ROY,7 AND V. REGGIE EDGERTON2,7

1Laboratory for Structure and Function Research, Division of Human Structure and Function, Departments of 1Physiology, 4Orthopedics, and 4Molecular Life Science, Tokai University School of Medicine, Bohséidai, Isehara, Kanagawa 259–1193; 2Tokai University School of Physical Education, Hiratsuka, Kanagawa 259–120, Japan; and 7Brain Research Institute and 2Department of Physiological Science, University of California, Los Angeles, California 90095

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AGING IS COMMONLY ASSOCIATED with a decrement in motor function due, in part, to a loss in muscular strength and fatigue resistance. In turn, these decrements are reflected in detrimental adaptations in some of the mechanical, biochemical, and morphological properties of the skeletal musculature (9, 12, 13, 20). Some of these adaptations appear to be related to a reduction in use, i.e., decreased levels of neuromuscular activity (26), as is typical of muscles in the lower extremities of old people and animals (9). These adaptations, however, also have been associated with intrinsic age-related changes in the muscles themselves (12).

Skeletal muscles in old individuals are responsive to increased levels of neuromuscular activity. For example, progressive resistance training results in strength gains due to either muscle hypertrophy (5, 25) and/or neural factors (24) in older people. The capacity for muscle hypertrophy is attenuated in older subjects (33). The reasons for the attenuated cellular and biochemical responses of atrophied muscles of older individuals to resistance exercise remain unclear. Heavy resistance exercise can induce inflammation and/or damage in the muscle, and the associated degeneration-regeneration responses can be beneficial for a subsequent increase in muscle mass (19). In addition, there appears to be an age-related impairment in myogenic potential as indicated by a deficiency in the capacity for skeletal muscle reinnervation in old rats (8, 23).

Heavy resistance exercise, such as weight lifting, is easy to apply in humans but is very difficult to apply in laboratory animals. Human studies on the cellular responses to resistance training are limited due to the invasive nature of muscle biopsies and to the risk of using old people as subjects, as well as the more limited control of lifestyles. To circumvent these problems, we have been using a weight-lifting protocol designed for rats (30). Recently, we have reported morphological and biochemical evidence of muscle fiber hyperplasia associated with muscle damage and regeneration in the plantaris (Plt) muscle after a single exhaustive session of weight lifting in previously nontrained young rats (28).

The purpose of the present study was to compare the myogenic response of hindlimb muscles in young (14–20 wk of age) and old (>120 wk of age) rats with a single exhaustive bout of heavy resistance weight lifting. [3H]thymidine and [14C]leucine labeling were monitored for up to 2 wk after the exercise bout to estimate serial changes in mitotic activity and the level of amino acid uptake and myosin synthesis. Histological, histochemical, and immunohistochemical [anti-5-bromo-2'-deoxyuridine and myogenic determination genes (MyoD)] analyses of whole muscles and analysis of muscle-specific gene expression (MyoD) using Western blotting and RT-PCR were performed. Old rats showed significant muscle atrophy and a lower exercise capacity than young rats. Exercise-induced muscle damage, as assessed in histological sections, and increases in serum creatine kinase activity were evident in both young and old exercised groups. Mitotic activity was increased in young, but not old, rats 2 days after exercise. There was a biphasic increase in [14C]leucine uptake during the 14 days postexercise (peaks at 1–4 and 10 days) in young rats: only the first peak was observed in old rats. There was a lower uptake of [14C]leucine in the myosin fraction and an impaired expression of MyoD at the protein (immunohistochemistry and Western blotting) and mRNA (RT-PCR) levels in old rats throughout the postexercise period. These results demonstrate a reduced reparative capability of muscle in response to a single bout of exercise in old compared with young rats.

satellite cell; mitotic activity; amino acid uptake; gene expression; myogenic determination gene; rat skeletal muscle
limb extensor and flexor muscles after the weight-lifting session were followed for 2 wk to determine the level of mitotic activity and amino acid uptake, respectively. The expression of myogenic determination genes (MyoD; protein and mRNA levels) and 5-bromo-2’-deoxyuridine (BrDU) labeling also were used as indicators of cell proliferation and cellular mitotic activity.

MATERIALS AND METHODS

Animals. The following two groups of Wistar male rats (specific pathogen free) were studied: 1) young (14–20 wk old; 380–520 g body mass; total n = 46) rats and 2) old (>120 wk old; 375–540 g body mass; total n = 37) rats. Exercised (see below) and nonexercised subgroups were studied in both age groups. In addition, 3-wk-old rats (85–95 g body mass; total n = 12) were used as positive controls for the analysis of muscle-specific gene expression (see below). The animals were housed in standard cages and were provided food and water ad libitum. The room temperature was kept at 23 ± 1°C, and a 12:12-h light-dark cycle was maintained throughout the experiment. All experimental procedures were conducted in accordance with the Japanese Physiological Society Guide for the Care and Use of Laboratory Animals as approved by the Tokai University School of Medicine Committee on Animal Care and Use and followed the American Physiological Society Animal Care Guidelines.

Exercise protocol. The hindlimbs of the rats in the exercise groups were trained for one intense exercise session using a weight-lifting technique described in detail elsewhere (28, 30). The exercise was performed preferentially by the plantarflexors, with a minimal usage of the dorsiflexors. The session involved multiple sets of 10 repetitions (lifts) per set with ~1 min rest between each set. The first set of lifts was with a 500-g load. In the subsequent sets, an additional 500-g load was added until the rat could not complete 10 repetitions. The load was then adjusted in 100-g increments and/or decrements until the maximum load at which 10 repetitions could be completed, i.e., the 10 repetition maximum (10 RM), was determined. The 10 RM was repeated until the rat could not complete the set, and then the load was decreased by 500 g. This procedure was followed until the rat failed to complete three consecutive sets even when the load was being reduced. The total time of the exercise bout was ~30–40 min for the young and ~20–30 min for the old rats. The 10 RM (g), number of sets, and total amount of load lifted (number of lifts × load lifted by different groups in absolute values expressed as kg) were recorded.

Measurement of serum CK activity. Blood samples (0.2 ml) were obtained from the caudal vein before and 30 and 60 min after the exercise session. Serum CK activity was measured using a standard kit (Monotest CK-NAC; Boehringer, Mannheim, Germany) and was used to estimate exercise-induced muscle damage. The activities were expressed as interna
tional units per milliliter.

Muscle fiber number. To determine whether a decrease in fiber number occurred during aging, the number of total and branched fibers of the PLT muscles in both groups were counted using a nitric acid digestion method (29, 30). Briefly, the frozen muscle was thawed in distilled water for a few minutes and then immersed in 15% nitric acid (Wako Pure Chemical, Osaka, Japan) for 2–3 h at room temperature. The muscle was washed in cooled distilled water for 60 min at 4°C. The solution was changed from distilled water to 0.01 M PBS (pH = 7.4) and was stored at 4°C. Subsequently, a few muscle fiber bundles were transferred to a silicone-coated petri dish containing 0.01 M PBS (pH = 7.4) at room temperature. All fibers were teased free, and the total number of straight (nonbranched) and branched fibers was carefully and precisely counted under a dissection microscope (×10–15). These procedures were repeated until all fibers, nonbranched and branched, in each muscle were counted.

Histochecmical and immunohistochemical analyses. Histochecmical and immunohistochemical analyses were performed 4 h after the exercise session in exercised (n = 4) and nonexercised (n = 2) rats in both groups. BrDU (Takeda Chemical, Osaka, Japan), a nonradioactive marker for DNA synthesis, was injected (100 mg/kg ip) 1 h before sampling. This procedure is useful for labeling proliferating cells in muscle (28, 29). The rats were killed with an overdose of pentobarbital sodium (60 mg/kg ip), and the soleus (Sol) and PLT muscles of both hindlimbs were excised within 10 min. The Sol and PLT muscles then were weighed after trimming excess connective tissue, were immediately frozen in isopentane precooled by liquid nitrogen, and were stored at −80°C. After equilibration at −20°C, each muscle was divided longitudinally into three (Sol) or six (PLT) portions to allow full characterization of the entire muscle (29). Serial 10-μm-thick transverse sections (10–20 sections) were cut from each muscle portion. Staining of hematoxylin-eosin and actomyosin ATPase (mATPase) after acid (pH 4.3) preincubation was performed on four to eight sections to examine the general morphology and the fiber-type characteristics of the muscles.

For the remaining 6–12 sections, immunohistochemical staining was performed using BrDU (monoclonal anti-BrDU from mouse; Becton-Dickinson, San Jose, CA) and MyoD (monoclonal anti-MyoD1 from mouse, 5.8A; Dako, Carpintia, CA) antibodies to identify and localize proliferating cells (BrDU) and myoblasts (MyoD) in the muscles.

For the BrDU staining, the sections were fixed in 70% ethanol for 15 min at 4°C and were treated with 100% methanol containing 0.3% H2O2 to inhibit endogenous peroxidase for 30 min at room temperature and then were treated with 2 N HCl for 60 min at room temperature for DNA denaturation followed by washing in 0.01 M PBS (pH = 7.4). The sections were treated with 10% normal sheep serum (NSS) in PBS for 30 min and were incubated for 60 min with the primary monoclonal antibody diluted 1:50 in PBS (pH = 7.4) containing 1% BSA (Seikagaku Kogyo, Tokyo, Japan). The sections were washed in PBS, treated with 10% NSS for 20 min, and incubated with anti-mouse IgG F(ab’)2, combined with sheep horseradish peroxidase (Amersham International, Buckinghamshire, UK) diluted 1:75 in PBS containing 1% BSA for 60 min at room temperature. Finally, the sections were washed in PBS, visualized with 0.02% 3,3-diaminobenzidine (Wako Pure Chemical)/0.05 M Tris–HCl buffer, pH = 7.4, containing 0.005% H2O2 for 5–10 min, and counterstained with eosin.

For the immunohistochemical staining of MyoD, the sections were fixed in 4% paraformaldehyde/0.05 M phosphate buffer (pH 7.4) for 15 min at room temperature. Anti-MyoD was used as the primary antibody diluted 1:50 in PBS containing 1% BSA. The same procedures described for the BrDU staining were used, except for the treatment of 2 N HCl.

Western blotting procedures. To determine the level of MyoD expression, the Sol and PLT muscles from two nonexercised rats from the young and old groups were prepared for Western blotting. In addition, muscles from two 3-wk-old rats were analyzed as a positive control. Each muscle was minced using scissors at 4°C on a ice-cooled glass plate. The samples were immersed in a homogenizing buffer containing 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS in
PBS, and 0.1 mM phenylmethylsulfonyl fluoride was added at the time of use at 4°C. Muscle tissues were homogenized (×10) with a glass homogenizer and were incubated for 30 min at 4°C. Each homogenate was transferred to microfuge tubes and centrifuged at 15,000 g for 20 min at 4°C, and the supernatant was obtained. Cell lysate with an equal volume of electrophoresis sample buffer (0.15 M Tris-HCl, 4% SDS, 10 mM EDTA, 40% glycerol, and 10% 2-mercaptoethanol, pH 6.7) was boiled for 5 min, separated by 10% SDS-PAGE, and blotted for 1.5 h with a constant current of 150 mA on a polyvinylidene difluoride membrane (ATTO, Tokyo, Japan). In this step, the total protein concentration of each sample was adjusted preliminarily. The membrane was blocked with a blocking buffer (5% nonfat milk powder in 0.01 M PBS, pH 7.4, containing 0.1% Tween 20) overnight at 4°C. Primary antibody [polyclonal anti-MyoD (C-20) from rabbit; Santa Cruz Biotechnology, Santa Cruz, CA] was applied in a dilution of 1:100 with blocking buffer for 1.5 h at 37°C. The blot was washed for 1.5 h with several changes of PBS containing 0.1% Tween 20 (PBS-Tween) at room temperature and then was incubated with a second antibody [anti-rabbit IgG F(ab')2, combined with sheep horseradish peroxidase; Amersham International] diluted 1:5,000 with blocking buffer for 1.5 h at 37°C. The blot was washed with PBS-Tween for 2 h. Antibody binding was visualized by the enhanced chemiluminescence (ECL) technique (ECL + plus System; Amersham International) and X-ray film (BioMax MS-1; Kodak, Tokyo, Japan). The immunoblots were photographed and scanned with a scanning densitometer (Ultrascan laser; Pharmacia LKB, Uppsala, Sweden) for quantification.

RT-PCR analyses. Total RNA was extracted from nonexercised young (n = 2), old (n = 3), and 3-wk-old (n = 10) rats using the guanidine isothiocyanate technique (10). The Sol and Plt muscles were dissected from each rat, the tendons were removed, and the muscles were minced using scissors at 4°C on an ice-cooled glass plate under the RNase-free condition. Each muscle was lysed in guanidine isothiocyanate buffer and homogenized using a polytron (Kinematica Littau/Luzen, Switzerland). The lysate was layered on a CsCl gradient buffer (5.7 M CsCl/3 M sodium acetate, pH 6, sterile), spun in an ultracentrifuge at 179,000 g (SW55; Beckman, Fullerton, CA) for 24 h at 20°C, and then prepared for extraction of intact total RNA. RNA quantity was determined by an optical density measurement at 260 nm. Samples were stored at −80°C. Primer pairs and the nucleotide position used for PCR amplification of MyoD mRNA were as follows: MyoD-forward, 5'-ACCATGGGAGCTATCTGCGC-GCA-3'; nucleotide 2590–2611 (32); MyoD-reverse, 5'-TCTCGGACCTGGTAAATCGGATT-3', complementary to nucleotide 4287–4307 (32). RT-PCR was performed using an RNA-PCR kit (Perkin-Elmer, Cetus, CT). To enable a comparison between muscles from young, old, and 3-wk-old rats, 25, 30, and 35 PCR cycles were chosen.

Analyses of mitotic activity and amino acid uptake. Our previous data indicate that in vivo[3H]thymidine and [14C]leucine labeling is a useful method to detect the mitotic activity and amino acid uptake in the muscles after the exercise (28). Analyses of mitotic activity and muscle amino acid uptake were performed at rest and 1–4, 7, 10, and 14 days after exercise in young rats (n = 31, 3–6 time point) and old rats (n = 24, 3 time point) rats. [3H]thymidine (methyl-[3H]; 15.5 MBq/kg ip; specific activity 247.9 GBq/mmol; NEN Life Science Products, Boston, MA) and [14C]leucine ([U-14C]; 1.15 MBq/kg ip; specific activity 13.6 GBq/mmol; NEN Life Science Products) were injected 1 and 3 h before sampling to label proliferating cells and proteins that use leucine during protein synthesis, respectively. The rats were overdosed with pentobarbital sodium (60 mg/kg ip), and the following muscles were removed bilaterally: primary extensors [Sol, Plt, and gastrocnemius (Gas)] and primary flexors [tibialis anterior (TA) and extensor digitorum longus (EDL)]. After excess connective tissue and fat were removed, each muscle was wet weighed, homogenized in guanidine isothiocyanate solution (Solvable; Packard, Meriden, CT) at 45°C, and then a material was treated overnight with 1 ml of dissolving material was collected and dried with 70% ethanol. The dried material was used for extraction of intact total RNA. Values are means ± SE; n, no. of rats. TA, tibialis anterior; EDL, extensor digitorum longus; Sol, soleus; Plt, plantaris; Gas, gastrocnemius. * Significant difference between young and old rats at P ≤ 0.01. The rats were overdosed with pentobarbital sodium (60 mg/kg ip), and the following muscles were removed bilaterally: primary extensors [Sol, Plt, and gastrocnemius (Gas)] and primary flexors [tibialis anterior (TA) and extensor digitorum longus (EDL)]. After excess connective tissue and fat were removed, each muscle was wet weighed and homogenized in 0.02 M phosphate buffer (pH = 7.4) at a 1:20 dilution at 4°C. In the next step, 1 ml of the homogenate from each muscle sample was added to 5 ml of 10% TCA and mixed well. This mixture was centrifuged (2,050 g for 10 min), and the upper solution (TCA) was removed. This procedure was repeated five times, and the remaining TCA-insoluble material was collected and dried with 70% ethanol. The dried material was treated overnight with 1 ml of dissolving solution (Solvable; Packard, Meriden, CT) at 45°C, and then a 10-mL liquid scintillation cocktail (Atomlight; Packard) was added to count radioactivity (Beckman LS4800). The total protein concentration in each homogenate was measured, and the radioactivity of each sample was expressed as disintegrations per minute per milligram protein.

We have previously reported that the uptake of thymidine and leucine into individual muscles within a rat and for an individual muscle across rats varies widely (28), most likely reflecting varying levels of recruitment of each muscle during the weight-lifting task. In all cases, the pooled values for the extensors (Sol, Plt, and Gas) had a higher amino acid uptake than the pooled values for the flexors (TA and EDL). Thus, to minimize the effects of intra- and intermuscle variability on the effects of exercise on thymidine and leucine uptake, the difference in the uptake between the extensor and flexor muscles in both legs of each rat is reported.

| Table 2. Number of total and branched fibers in the plantaris muscle of young and old rats |
|-----------------|-----------------|
| Young (n = 5)   | Old (n = 3)     |
| Total fiber number | 11,284 ± 369*  | 9,370 ± 135         |
| Number of branched fibers | 29 ± 4          | 36 ± 1               |

Values are means ± SE (n, no. of rats) and are derived from nonexercised rats in both age groups. The decrease in muscle fiber number may be a reflection of an age-associated decrease in the total number of motor units, presumably of the fast type (18). * Significant difference between young and old rats at P ≤ 0.01.
Analysis of myosin synthesis. The determination of myosin synthesis was performed at the same time points and for the same groups as for the analysis of mitotic activity and amino acid uptake. Myosin was extracted with 0.6 M KCl solution (50 ml) from a 1-ml homogenate for 15 min at 4°C and was filtered with three sheets of gauze. The myosin-extracted KCl solution was diluted with cool distilled water (1:20), which resulted in the reappearance of myosin deposits. The diluted solution was passed through an omnipore nondissolving membrane filter (10 µm aperture and 47 mm diameter; Nihon Millipore, Yonezawa, Japan). The membrane containing the deposits was dried, cut into several pieces, and soaked in a dissolving solution overnight at 45°C. The radioactivity was counted using the same procedures employed for the mitotic activity and protein synthesis analyses. Values were expressed as disintegrations per minute per milligram protein.

Statistical analyses. All data are expressed as means ± SE. Differences in body mass, muscle mass, fiber numbers, and [3H]thymidine and [14C]leucine uptake levels between young and old rats were determined using Student’s t-tests. ANOVA was used to determine overall differences, and Duncan’s post hoc analyses were used for individual group differences for the preexercise and serial postexercise data for [3H]thymidine, [14C]leucine, and myosin fraction uptake. Standard regression analysis and Pearson’s product correlation procedures were used to determine the relationship between [3H]thymidine uptake and CK activity. Differences were considered statistically significant at either the 0.05 or the 0.01 levels.

RESULTS

Exercise capacity. The total load lifted during the single exhaustive exercise bout was lower in old than young rats. The total number of sets, the 10 RM, and the total amount of load lifted were 24 ± 3 and 13 ± 5 sets, 2,664 ± 38 and 2,083 ± 42 g, and 417 ± 86 and 209 ± 26 kg for the young and old rats, respectively. All values were significantly higher in the young than the old rats (P < 0.01).

Body mass, muscle mass, and total muscle fiber number. The mean final body mass was not significantly different between the two groups (Table 1). However, it should be noted that the body masses of the old rats had increased to 650–750 g and then gradually decreased with advancing age. The mean absolute masses of the TA, Plt, and Gas and relative masses of TA, Sol, Plt, and Gas were significantly smaller in old than young rats. The mean total fiber number in the Plt muscle was significantly lower (17%) in old than young rats (Table 2). Although the absolute number of branched fibers was 24% (P > 0.05) higher in the old than the young rats, the incidence of branched fibers relative to nonbranched fibers was low in both young (0.26%) and old (0.38%) rats.

Fig. 1. Mean serum creatine kinase (CK) activities at rest and 30 and 60 min after a single exhaustive resistive bout of exercise. Bars are SE. IU, international units. Rest, preexercise. *P < 0.05 (young vs. old rats); †P < 0.05 and ††P < 0.01 (rest vs. postexercise).

Fig. 2. Representative cross-sectional profiles of the plantaris muscles of a young (A) and an old (B and C) rat. A and B: actomyosin ATPase at a preincubation of pH 4.3. C: hematoxylin and eosin (HE) staining. Fiber type grouping (B) and small angulated fibers (arrowheads in C) were evident in muscles of old, but not young (A), rats. The appearance of small angulated fibers is consistent with degenerating fibers that are not reinnervated (3, 21, 22). Fiber type grouping of slow fibers in older animals (14) has been interpreted as evidence for the reinnervation of some of the fast fibers by motoneurons innervating slow muscle fibers (12, 16). Calibration bar is equal to 100 µm. Magnifications: A and B, ×24; C, ×120.
Serum CK activity. The mean preexercise serum CK levels were similar in young (n = 25) and old (n = 21) rats (Fig. 1). These levels were significantly increased in both groups 30 or 60 min after the exercise session. The postexercise values were significantly lower in the old than the young rats at both time points.

Muscle morphology and immunostaining. In contrast to the typical mosaic pattern of fiber types (slow fibers dark, based on mATPase staining at a pH 4.3 preincubation) in the Plt muscle of young rats (Fig. 2A), “fiber type grouping,” i.e., an abnormal number of fibers of the same ATPase type that are adjacent, was observed in the Plt of old rats (Fig. 2B). In addition, fibers having unusual cross-sectional shapes, e.g., small angulated fibers (Fig. 2C), were evident in all muscles of old, but not young, rats.

Necrotic fibers were scattered throughout the Plt muscle cross section 2 days after exercise in both young (Fig. 3A) and old (Fig. 3B) rats. The overall mean number of these necrotic fibers for both exercised groups was 29 ± 10 per cross section, i.e., occupying ~0.5–0.6% of the cross section. Note that there were almost no necrotic fibers in young nonexercised rats and only a few in old nonexercised rats.

Immunostaining patterns for anti-BrDU (Fig. 3, C and D) and anti-MyoD 2 days after the exercise session are shown for the Plt (Fig. 3, E and F) and Sol (Fig. 3, G and H) muscle of a representative young (Fig. 3, C, E, and G) and old (Fig. 3, D, F, and H) rat.
An increase in proliferating cells occurred in and/or near necrotic fibers and around some normal-appearing fibers in the muscle of young rats (Fig. 3C). Only one or two anti-BrDU positive cells were observed near the necrotic fibers in the muscles of old rats (Fig. 3D). Similar differences between the two age groups were observed for anti-MyoD staining. Generally, in the young rats, the localization and number of anti-MyoD positive cells were similar to that of the anti-BrDU positive cells. A large number of MyoD positive cells was observed in the Plt of young rats (Fig. 3E), but only a few were seen in old (Fig. 3F) rats. This tendency was similar in the Sol muscles of young (Fig. 3G) and old (Fig. 3H) rats. Positive reactions were rarely observed in nonexercised rats of either age group. Edema (increased extracellular space) after weight-lifting exercise was evident histologically in the muscles of both young and old rats.

Expression of MyoD protein and mRNA. An immunoreactive band of 35 kDa corresponding to the predicted molecular mass of the rat MyoD protein (Fig. 4). The lowest levels of MyoD positive cells were observed in both the Sol and Plt of old rats. To examine the age dependence of the MyoD expression, we compared the nonexercised young and old rats with 3-wk-old rats. MyoD protein tended to be higher in the 3-wk-old than in the young rats for the Plt but not the Sol muscles. The expression of MyoD mRNA levels in both the Sol and Plt was highest in the 3-wk-old rats and lowest in the old rats (see 25 and 30 cycles; Fig. 5). The expression levels were lowest in the Sol of old rats (Fig. 4). It also

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Values are means ± SE expressed as dpm/mg protein; n, no. of rats. Flexors, tibialis anterior and extensor digitorum longus; extensors, soleus, plantaris, and gastrocnemius. *Significant difference between young and old rats at P < 0.05.

![Image](http://ajpcell.physiology.org/)
appeared that the expression of MyoD mRNA levels were higher in the Plt than the Sol muscle in all groups. We confirmed that the nucleotide sequence of the RT-PCR product was completely matched to the MyoD mRNA (data not shown).

Thymidine and amino acid uptake rates. The flexor muscles (TA and EDL) of old rats had a significantly lower uptake of \([^{3}H]\)thymidine compared with young rats in the resting state (Table 3). The extensor muscles (Sol, Plt, and Gas) in young and old rats were similar in thymidine uptake. There was no significant difference between young and old rats in \([^{14}C]\)leucine uptake in either the extensor or flexor muscles. Significant increases in the uptakes of \([^{3}H]\)thymidine and \([^{14}C]\)leucine in the extensors of young rats were observed at 2 and 10 days after exercise, respectively, whereas there was no significant change in the extensors of the old rats (compare Figs. 6 and 7). In addition, no significant changes in the uptake of either \([^{3}H]\)thymidine or \([^{14}C]\)leucine were observed in the flexor muscles of young or old rats after the single bout of exercise (Figs. 6 and 7). Because there was no exercise effect on the overall mean uptakes for the flexor muscles for either group (Figs. 6 and 7), we then normalized the exercise effects of the extensors to the flexor muscles and compared the response of young and old rats (Fig. 8). This procedure minimized the intra-animal variability. The uptake of \([^{3}H]\)thymidine was elevated significantly at 2 days after exercise in young rats (Fig. 8A). In contrast, there was a small insignificant peak at 1 day after exercise in old rats (Fig. 8A). \([^{14}C]\)leucine uptake in the young rats was elevated up to 10 days after exercise (significant differences at 3 and 10 days) and then returned to rest levels at 14 days. In the old rats, the uptake pattern was similar to that of the young rats for up to 7 days but returned to resting levels 10 days after exercise and remained at that level up to 14 days (Fig. 8B).

Fig. 7. Absolute change in \([^{3}H]\)thymidine (A) and \([^{14}C]\)leucine (B) uptake in extensors and flexors during the 2 wk after a single exhaustive bout of weight lifting in old rats. Values are expressed as means ± SE. Extensors, Sol, Plt, and gastrocnemius; flexors, tibialis anterior and extensor digitorum longus. Rest, values from nonexercised control rats. Note that there are no significant changes during the 2 wk after the exercise bout.

Fig. 8. Changes in \([^{3}H]\)thymidine (A) and \([^{14}C]\)leucine (B) uptake during the 2 wk after a single exhaustive bout of weight lifting. Uptake values were calculated as (mean value of extensors) - (mean value of flexors) in both legs and are expressed as means ± SE. Extensors, Sol, Plt, and gastrocnemius; flexors, tibialis anterior and extensor digitorum longus. Rest, values from nonexercised control rats. * and ** Significantly different from rest at \(P < 0.05\) and \(P < 0.01\), respectively.
Fraction of amino acid uptake for myosin. The uptake of $[14C]$leucine for the myosin fraction for the extensor muscles was elevated significantly at 10 days after exercise for the young rats, whereas there were no changes at any other postexercise time point for the young or old rats (Fig. 9A). When the uptake of $[14C]$leucine for the myosin fraction on the extensor muscles was expressed relative (%) to total uptake, there was a progressive decrease in old rats that reached significance at 7, 10, and 14 days postexercise (Fig. 9B). The resting levels for the relative uptake of $[14C]$leucine for the myosin fraction were similar in the young and old rats, i.e., 29 ± 3 and 26 ± 4%, respectively.

**DISCUSSION**

Muscle damage and appearance of proliferating cells and myoblasts. Although the serum CK activities 30–60 min after the exercise bout were increased significantly in both the young and old rats, there were no changes at any other postexercise time point for the young or old rats (Fig. 9A). When the uptake of $[14C]$leucine for the myosin fraction on the extensor muscles was expressed relative (%) to total uptake, there was a progressive decrease in old rats that reached significance at 7, 10, and 14 days postexercise (Fig. 9B). The resting levels for the relative uptake of $[14C]$leucine for the myosin fraction were similar in the young and old rats, i.e., 29 ± 3 and 26 ± 4%, respectively.
An impaired capacity for muscle regeneration in old animals also has been reported after bupivacaine injection (8), contraction-induced injury (12), ischemic necrosis (31), eccentric contraction-induced injury (4), and transplantation of muscle grafts (7).

The more limited myogenic potential of muscles in old rats may reflect a more limited plasticity of the nervous system. Carlson and Faulkner (6, 8) suggested that a reduced capacity for reinnervation in muscles of old rats may be a contributing factor to the decreased muscle regenerative capacity. Innervation is an essential factor for the differentiation from a myotube to a myofiber during muscle regeneration (1). The results of the present study clearly demonstrate that the impaired regenerative capacity of muscles in the old rats was associated with a reduction in, or the lack of, proliferating capability of the satellite and/or stem cells.

In contrast to the in vivo studies noted above, some tissue culture studies suggest that satellite cells from muscles of old rats maintain the capacity to replicate (27, 34) and that the rate of proliferation is not decreased with age (11, 17). These cells in culture appear to have only an increased “lag phase” before the onset of proliferation (11, 17, 27). These differences in results obtained from in vivo and in vitro studies emphasize the importance of taking into account the experimental paradigms when comparing the responses of cellular and system studies.

Amino acid uptake and ability for muscle hypertrophy. Young rats showed a biphasic response in [14C]leucine uptake, i.e., peaks at 1-4 and at 10 days after the exercise bout (Fig. 8B). The first peak most likely reflects increased protein synthesis (hypertrophy) in the primary tissues (contractile proteins, connective tissue, revascularization, activation of satellite cells, etc.), whereas the second peak may reflect the synthesis of the contractile components of regenerated and/or de novo muscle fibers (hyperplasia; see Ref. 28). Previous studies have demonstrated that muscle protein synthesis accelerates after the formation of myotubes to allow for accumulation of the contractile components within the cytoplasm and differentiation of the myofibers (15). The old rats showed a similar peak in [14C]leucine uptake at 1 wk but no peak at 2 wk after the exercise bout (Fig. 8B). Furthermore, the postexercise data for [14C]leucine uptake for myosin (Fig. 9A) in the extensor muscles and for the fraction of myosin uptake relative (%) to the total uptake (Fig. 9B) suggest that the increase in amino acid uptake during the first week in old rats was used primarily for the synthesis of noncontractile protein such as connective tissue. An age-related decrease of myosin heavy chain synthesis rate has been reported by Balagopal et al. (2), suggesting a decreased capacity for hypertrophy in muscles of old rats. Combined with the small increase in [3H]thymidine uptake in old rats, these results support the view that there is a lower muscle capacity for hypertrophy, including hyperplasia, in old than in young rats.

Perspective

There was a lower reparative capacity of skeletal muscles in older than in younger rats that was related to a reduction in myogenic cell (satellite cell) activity in vivo. Most of the myogenic cells in the muscles of old rats did not enter the cell cycle, based on the low number of BrDU and MyoD positive cells after the weight-lifting exercise bout. This loss of myogenic cell activation and proliferation potential may be one of the critical factors in the reduction of the function of skeletal muscle in the later stages of life. Although the reduced effects of the exercise bout in old rats could have been due to a lower level of overload in old compared with young rats, this is unlikely for two reasons. First, markers for “muscle damage” were significantly elevated in old rats after the exercise bout. Second, the same criteria for exhaustive exercise was used for both young and old rats. Thus it appears that not only does the muscle in the aged rat have a reduced performance potential but its ability to respond to functional perturbations and maintain a homeostatic state is markedly reduced.

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Address for reprint requests and other correspondence: T. Tamaki, Dept. of Physiology, Division of Human Structure and Function, Tokai Univ. School of Medicine, Bohsedei, Isehara, Kanagawa 259–11 Japan (E-mail: tamaki@is.icc.u-tokai.ac.jp).

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