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Fiber-type susceptibility to eccentric contraction-induced damage of hindlimb-unloaded rat AL muscles

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Vijayan, K., J. L. Thompson, K. M. Norenberg, R. H. Fitts, and D. A. Riley. Fiber-type susceptibility to eccentric contraction-induced damage of hindlimb-unloaded rat AL muscles. *J Appl Physiol* 90: 770–776, 2001.—Slow oxidative (SO) fibers of the adductor longus (AL) were predominantly damaged during voluntary reloading of hindlimb unloaded (HU) rats and appeared explainable by preferential SO fiber recruitment. The present study assessed damage after eliminating the variable of voluntary recruitment by tetanically activating all fibers in situ through the motor nerve while applying eccentric (lengthening) or isometric contractions. Muscles were aldehyde fixed and resin embedded, and semithin sections were cut. Sarcomere lesions were quantified in toluidine blue-stained sections. Fibers were typed in serial sections immunostained with antifast myosin and antitotal myosin (which highlights slow fibers). Both isometric and eccentric paradigms caused fatigue. Lesions occurred only in eccentrically contracted control and HU muscles. Fatigue did not cause lesions. HU increased damage because lesioned-fiber percentages within fiber types and lesion sizes were greater than control. Fast oxidative glycolytic (FOG) fibers were predominantly damaged. In no case did damaged SO fibers predominate. Thus, when FOG, SO, and hybrid fibers are actively lengthened in chronically unloaded muscle, FOG fibers are intrinsically more susceptible to damage than SO fibers. Damaged hybrid-fiber proportions ranged between these extremes.

skeletal muscle; missing A-band lesions; motor unit recruitment; hybrid fibers; reloading; adductor longus

EXTENDED PERIODS OF SKELETAL muscle unloading, such as chronic bed rest and spaceflight for humans and spaceflight and hindlimb unloading (HU) for rodents, increase susceptibility to reloading injury (10, 18, 20, 21, 23, 24, 26, 28). Returning the rats to gravity loading predominantly damaged fibers in the slow-twitch adductor longus (AL) muscles (20, 21, 23). A variety of studies in humans and animals indicate that exercise injury can be slow or fast fiber type dominant (1, 9, 12, 13). In these studies, injury was elicited by eccentric contractions in which muscles are lengthened while actively generating tension. In humans, type II fibers were injured in the calf muscles during backward walking on an inclined treadmill (9). Conversely, rats running downhill on a treadmill showed damage predominantly in slow-twitch muscles (1). For rat HU, we reported that slow oxidative (SO) fibers were preferentially damaged after reloading (26). Whether particular fiber types are intrinsically more vulnerable to injury is unclear because uncontrolled variables, such as motor unit recruitment, may determine the pattern of damage (2, 3, 23). During normal recruitment, lower threshold slow motor units are activated earlier than higher threshold fast motor units (2, 3). Glycogen depletion studies in humans indicate that slow fibers are utilized during submaximal endurance exercise and fast fibers are recruited after slow fibers are depleted of glycogen. Exercise exceeding maximal aerobic power utilizes both fiber types (6). Fibers must be actively contracting to experience injury, because inactive fibers are not damaged by lengthening that does not exceed thick and thin filament overlap (12, 15, 17). A comparison of damage susceptibility of isolated and permeabilized single muscle fibers showed greater tension deficits after eccentric contraction, i.e., lengthening active fast fibers from rat fast extensor digitorum longus (EDL) and slow soleus muscles (14). To explore the issue of intrinsic fiber-type susceptibility in unloaded muscles without the uncontrolled variable of recruitment, all of the fibers in 14-day HU rat AL muscles were activated in situ by tetanic electrical stimulation and subjected to controlled eccentric (lengthening) loading.

The basis for increased susceptibility after unloading is not well understood, but conversion of slow fiber properties toward fast fibers may reflect shifting from an injury-resistant to an injury-susceptible fiber type. Conversion is frequently incomplete, generating hybrid fibers that coexpress slow and fast muscle protein isoforms (26). The heterogeneous hybrid fibers may be the most damage-susceptible class because of suboptimal matching of protein properties and physiological demands, leading to sarcomere instability (7). Normal rat AL muscles contain SO and fast oxidative glycolytic (FOG) fiber types. Hybrid fibers, which are rare in normal AL muscles, were induced to ~29% of the population by 14-day HU (26). The degree of loading

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strain applied was chosen to be minimally damaging for normal muscles but destructive of unloaded muscles, because this avoided exceeding the damage threshold for the majority of fibers in the HU rats and revealed a graded response of fiber types to injury. The strain was applied in a series of eccentric contractions over a 5-min period. This protocol was chosen to simulate the injury that results from repeated contractions during voluntary reloading (24, 26), as well as to ensure that sufficient damage occurred across fiber types so that a statistical comparison of fiber-type damage could be made.

METHODS

Animals

Nine male Sprague Dawley rats (300–400 g) were housed in standard vivarium cages as normally loaded controls, and another nine were HU for 2 wk using the tail-harnessing method of Riley et al. (21, 22). Briefly, vinyl cloth strips were stuck to the tail with rubber cement and reinforced by wrapping with Elastoplast tape. A nylon fish line was secured to an overhead rod and tied to a wire attached to the vinyl harness. A nylon line was attached to the distal end of the harness. The length of the line was adjusted daily to suspend the rat and prevent HU. Animals were checked three times per day and cleaned of urine and feces as needed to assist grooming. Food and water were provided ad libitum. Procedures complied with protocols approved by the Animal Care Committees at Marquette University and the Medical College of Wisconsin.

In Situ Muscle Eccentric and Isometric Contractions

The whole muscle tetanic stimulation and lengthening procedures were modeled after those of Thompson et al. (24). The left AL muscle was exposed in a rat that was anesthetized with pentobarbital sodium (50 mg/kg body wt ip). The distal tendon was severed and tied with a silk suture to the servomotor arm of a Cambridge force transducer (model 352, Cambridge Technology, Cambridge, MA). The servomotor arm provided either isometric contraction or eccentric (active lengthening) contraction under computer control. The starting temperature of the muscle was maintained at 35–36°C by enclosing the preparation in a 40°C chamber and dripping warm Ringer solution on the muscle, which also prevented drying. AL muscles were fully activated by indirect stimulation through plate electrodes (1.5-mm wide) on the superficial and deep surfaces of the muscle at the end-plate region where the nerve enters the muscle. Peak isometric twitch tensions were elicited with 1- to 1.5-V stimulation for all muscles. This voltage was doubled for supramaximal stimulation, and increasing it above 3 V did not elicit higher twitch tensions. Muscle length was further adjusted to achieve maximum isometric twitch tension and to define optimum resting length (L_o). Maximum tetanic contractions were induced with 100-Hz square-wave pulses (11). Specific tension was calculated as maximum tetanic tension per milligram of muscle wet weight. The right AL served as a sham preparation, except that the distal tendon was not cut and the muscle was not stimulated.

For lengthening during contraction, a strain equal to 15% of L_o, beginning at L_o, was chosen because it falls within the physiological range for soleus, a slow muscle of similar fiber-type composition (14, 27). One hundred cycles of eccentric contractions were elicited in a 5-min period for five normal rats and five 14-day HU rats. Each cycle consisted of tetanic stimulation for 350 ms, continuing stimulation and lengthening for 500 ms, and halting stimulation and allowing the muscle to return to L_o over 500 ms (Fig. 1). This sequence was followed by a 1,650-ms rest. Cycles were repeated every 3 s for 5 min. Prelengthening tetanic tension was measured at 350 ms into *cycle 1*, and posttreatment tetanic force was measured at the same time point for the last cycle, *cycle 100*, to determine the degree of tension decline during stimulation

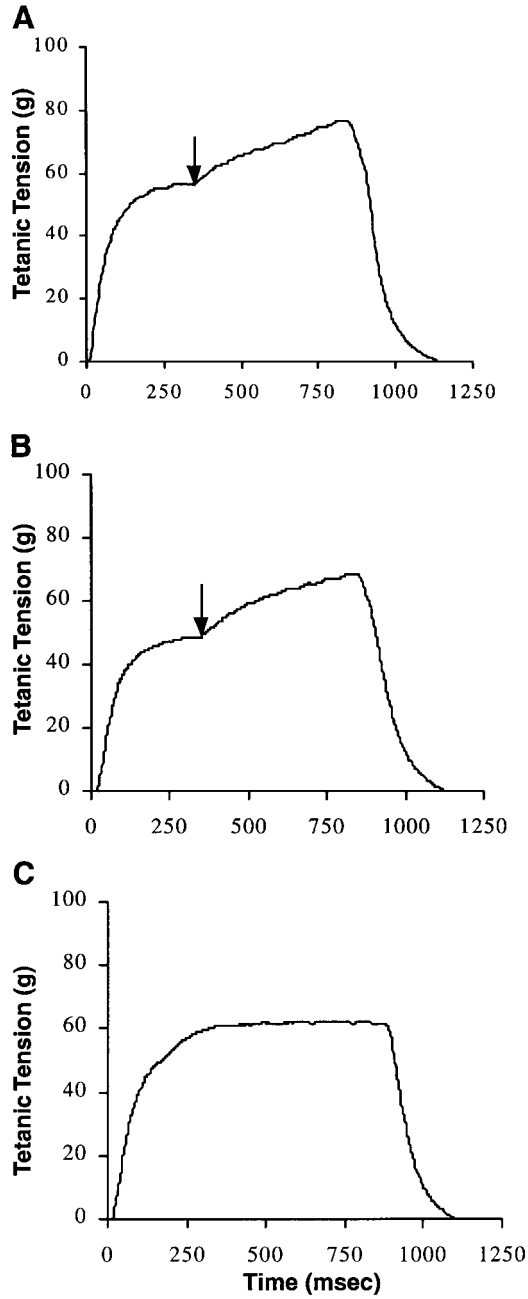

Fig. 1. Representative tetanic tension traces illustrate the effects of stimulation at 100 Hz in 14-day hindlimb unloaded (HU) rats for *cycle 1* (*A*) and *cycle 100* (*B*) of the 5-min eccentric contraction protocol. Arrows indicate isometric tension developed at the onset of lengthening, after 350 ms within each cycle. Comparing these time points shows a reduction in tension that is, presumably, muscle fatigue related. Tetanic tension fully recovered 10 min after completion of *cycle 100* and cessation of eccentric contraction treatment (*C*).

(Fig. 1). Isometric tetanic tension was checked 10 min later to evaluate recovery (Fig. 1).

In four control and four HU rats, 100 isometric contraction cycles were elicited over a 5-min period. The muscles were tetanically stimulated for 850 ms at L_o without lengthening. Pre- and posttetanic tensions were measured at 350 ms in both *cycles 1* and *100* to assess the decrease in tension.

Tissue Processing

After stimulation, muscles were excised, pinned straight at L_o, and immersion fixed in 1% paraformaldehyde, 0.05% glutaraldehyde, and 0.1 M sodium cacodylate buffer (pH 7.2), as previously described (26). Fixed muscles were rinsed in buffer, blotted, weighed, and returned to fresh buffer overnight at 4°C (26). The proximal one-third of each muscle was removed, cryoprotected in phosphate buffer containing increasing sucrose concentrations (10, 20, and 30%), and quick frozen in Freon-22 cooled in liquid nitrogen. The remaining two-thirds were dehydrated in graded ethanols, infiltrated, and embedded in hard grade LR White resin (Ted Pella, Costa Mesa, CA).

Lesion Detection and Fiber-type Identification

Oblique, semithin (0.5 μm) sections of fibers were cut from LR White resin blocks from the rostral region of AL containing SO and FOG fibers (21). Depending on the muscle fiber orientations, the number of fibers per section averaged 256 ± 130 (SE) and ranged from 41 to 423 fibers. The more longitudinally oriented (less oblique) sections contained the fewest numbers of fibers. Sections were stained with 0.5% toluidine blue dye in borax to highlight cross striations. Sarcomere lesions appeared as A-band disruptions (missing A bands) in toluidine blue sections (Fig. 2). Multiple toluidine blue-stained survey sections were screened for representative lesion damage in muscle samples (24–26). When a representative lesion area was located, a set of serial sections [one for histology and four for immunostaining (two primary with secondary and two secondary-only antibody stains)] were obtained. All fibers in the histology section were classified as lesioned or nonlesioned. The serial sections were fiber typed by indirect immunofluorescence using the primary antibody-to-total sarcomeric myosin ratio (M7523, 1:20, Sigma Chemical) and the primary antibody-to-fast myosin ratio (M4276, MY-32, 1:300, Sigma Chemical), as described previously (24–26). M7523 stains SO fibers more intensely than FOG fibers, and M4276 stains FOG fibers but not SO fibers (25, 26). Greater resolution of fiber types was vigorously pursued, but none of a large series of slow myosin antibodies or fast myosin isoform (IIa, IIb, IIb/IIx) antibodies tested (which stain fiber types in unfixed, frozen cryostat sections) worked in LR White sections (Ref. 25 and D. A. Riley, J. L. Thompson, and K. Vijayan, unpublished observations).

Fig. 2. Sarcomere lesions (arrows) induced by lengthening stress during eccentric contractions appear as missing A bands in the toluidine blue-stained sections. On average, lesions in control muscles (*A*) involved ~43% fewer sarcomeres in series than lesions in HU muscles (*B*). Scale bar = 8 μm.

Analysis of sarcomere lesion damage. The incidences of lesioned fibers were always compared within a section because lengthening deformation was presumably more uniform for contiguous fibers than for fibers in different sections. Lesion size was determined as the number of consecutively disrupted sarcomeres per lesion. Average lesion size, defined as the number of consecutively disrupted sarcomeres, was compared for muscles from normal and HU rats using the Mann-Whitney *U*-test (Ref. 32; Kaplan Statistics v 2.0). Significance was accepted at the $P < 0.05$ level. Values are presented as means ± SE. The fiber-type identities of lesioned and nonlesioned fibers were determined by identifying the same fibers in the toluidine blue and immunostained serial sections (25, 26). SO fibers (total myosin positive, fast myosin negative), FOG fibers (total negative, fast positive), and hybrid fibers (total and fast positive) were classified in photographic montages (×62.5 final magnification). For each HU rat, a 2 × 3 contingency table of lesion occurrence (lesioned, nonlesioned) vs. fiber type (SO, hybrid, FOG) was constructed. Independence of lesion occurrence and fiber type was tested via the G statistic (G_{stat}) and the null hypothesis (FOG = hybrid = SO) for a contingency table and was rejected at the $P \leq 0.05$ level ($G_{stat} \geq \chi^2_{crit.\ 0.05,\ v=2} = 5.991$) (BIOM Statistical Software Package; Applied Biostatistics, Setauket, NY). Nonsignificant subsets within each 2 × 3 table were identified as those with $G_{subset} \leq \chi^2_{crit.\ 0.05,\ v=2} = 5.991$. Averaged percentages of lesioned FOG, SO, and hybrid fibers were calculated for the HU group and reported with the SE. The averages were compared using the Kruskal-Wallis H test to determine significant differences at $P < 0.05$. This was followed by a multiple-range test by ranks to determine which subgroups (SO, FOG, or hybrid) were different from each other. For each of the control rats, a 2 × 2 contingency table was constructed, because only FOG and SO fibers were present in four of five rats. The properties of fiber type (FOG vs. SO) and fiber damage (lesioned vs. nonlesioned) were tested via the Fisher exact test (32). The null hypothesis was rejected at the $P \leq 0.05$ level. The Fisher exact test was used because of biasing in the χ^2 test due to lower lesion frequency in the control animals. Average percentages of lesioned SO and FOG fibers were calculated for the control group and compared using the Mann-Whitney *U*-test to determine significant differences at $P < 0.05$ (32). Average percentages of lesioned SO and FOG fibers for the control group were compared with average percentages of lesioned SO and FOG fibers in the HU group using the Mann-Whitney *U*-test, and significance was accepted at $P < 0.05$ (32).

Muscle Histology

Cross sections (10 μm) of the frozen proximal portions of AL muscles were cut with a cryostat microtome and stained with hematoxylin and eosin dyes. The sections were examined for general tissue morphology and signs of inflammation, by way of neutrophil invasion, in response to injury.

RESULTS

Muscle Weight and Force

The mean body weights of the control (376 ± 34 g) and 14-day HU (359 ± 34 g) rats were similar. Average AL muscle weight-to-body weight ratio for HU rats (0.18 ± 0.02 mg/g) was 42% less than control (0.31 ± 0.04 mg/g). Consistent with this lower mass, mean tetanic force decreased by 41% (106.7 ± 5.0 vs. 63.4 ± 7.1 g for control and HU, respectively). Atrophy did not alter average specific tension, defined as maximum tetanic tension divided by muscle weight (control 0.9 ± 0.1 vs. HU 1.0 ± 0.1 g/mg). Force could not be normalized to fiber area, as originally intended, because the cross-sectioned fibers in the hematoxylin- and eosin-stained sections were irreversibly shrunken osmotically during freezing, despite cryoprotection. The oblique plastic sections were optimal for detecting lesions but inappropriate for measuring fiber areas.

Eccentric and Isometric Contractions

Tetanic tension declined during the 5-min stimulation treatments (Fig. 1, *A* and *B*). In the eccentric contraction groups, tension fell 30 ± 19% for the HU and control rats. This fall in tension appeared to be fatigue rather than permanent damage because, 10 min after cessation of stimulation, force almost fully recovered (98 ± 7%) to prestimulation levels (Fig. 1*C*). Isometric stimulation caused similar tension declines during the 5-min treatment but, notably, did not induce sarcomere disruptions. However, lesions were common after the eccentric protocol.

Sarcomere Lesions and Fiber Type

Missing A-band lesions (24) involved one to several consecutive sarcomeres and occurred in eccentrically contracted AL muscles from control and HU rats (Fig. 2). In control rats, ~5% of all fibers sampled (n = 1,295) were lesioned. The percentage of all sampled fibers (n = 1,266) lesioned in the HU rats was ~15%. The average lesion size in HU rats (7.0 ± 2.8 disrupted sarcomeres/lesion) was significantly ($P < 0.05$) greater than that in control rats (3.0 ± 1.0 disrupted sarcomeres/lesion; see Fig. 2). None or very few lesioned fibers (1 ± 1%) were detected in the sham-treated muscles from control and HU rats. In contrast to the high occurrence of lesions after eccentric contraction, the percentage of lesioned fibers after isometric contraction was at background levels in control rats (2 ± 1%) and undetectable in HU rats. The Fisher exact test demonstrated that lesion frequency and fiber type were independent in eccentrically contracted AL muscles of control *rats 1*, *3*, and *5* (Table 1). Lesion frequency and fiber type were not independent in AL muscles of control *rats 2* and *4*, with FOG fibers being preferentially damaged over SO fibers (Table 1). In no instance were SO fibers preferentially damaged in control rats. The average percentage of lesioned SO fibers (1 ± 1%) was not significantly different from the percentage of lesioned FOG fibers (9 ± 3%) for the control group ($P < 0.075$).

In the eccentrically challenged HU muscles, lesion occurrence and fiber type were not independent ($P \leq 0.016$). For all five HU muscles, the G_{subset} statistic indicated that the proportion of lesioned FOG fibers was significantly greater than the expected value, whereas proportions of lesioned SO fibers were always significantly less than the expected values. Interestingly, the proportion of lesioned hybrid fibers varied the most, ranging widely between the most damaged (FOG) and the least damaged (SO) fiber types (Table 2). The average percentage of lesioned FOG fibers (44 ± 15%) in the HU group was significantly higher than the percentages of lesioned SO (11 ± 3%) and hybrid fibers (15 ± 4%) ($P < 0.01$). However, the average percentage of lesioned SO fibers was not significantly different from the percentage of lesioned hybrid fibers (15 ± 4%). The average percentage of lesioned SO fibers in the HU group was also not significantly different from the average percentage of lesioned SO fibers in the control group. The average percentage of lesioned FOG fibers in the HU group (44 ± 15%) was significantly higher than the average percentage of lesioned FOG fibers in the control group (9 ± 3%).

Table 1. *Sarcomere lesions in fibers of control rats*

	SO Fibers			FOG Fibers			
Rat No.	No. fibers sampled	Lesioned/ total SO	%SO lesioned	Lesioned/ total FOG	%FOG lesioned	Contingency Table Analysis, Probability Reject Null Hypothesis	2×2 Test of Independence, Fiber-type Susceptibility*
1	103	1/62	2	0/35	0	0.639	S≅F
2	357	0/216	0	17/141	12	0.0001†	S<F
3	85	0/18	0	8/67	12	0.136	S≅F
4	327	8/214	4	21/113	19	0.0000†	S<F
5	423	1/391	0	1/32	3	0.146	S≅F
Mean ± SE	259 ± 69		1 ± 1		9 ± 3‡		

SO, slow oxidative fibers; FOG, fast oxidative glycolytic fibers; S, SO fibers; F, FOG fibers. *Fisher exact test was used to determine whether fiber type (FOG vs. SO) and fiber damage (lesioned vs. not lesioned) were independent. The null hypothesis of independence (S = F) was rejected at $P < 0.05$. †2 of the control animals were significant. Semithin section examined from the eccentrically contracted adductor longus muscle of *rat 1* had 6 hybrid fibers in addition to slow and fast fibers; none of the hybrid fibers was lesioned. ‡Lesioned fast fiber percentage was not significantly different from lesioned slow fiber percentage ($P \leq 0.075$); F ≥ S.

Table 2. *Sarcomere lesions in fibers of HU rats*

	SO Fibers			Hybrid Fibers		FOG Fibers		Contingency Table Analysis, Probability Reject Null Hypothesis	2×3 Test of Independence, Fiber-type Susceptibility*
Rat No.	No. fibers sampled	Lesioned/ total SO	%SO lesioned	Lesioned/ total hybrid	%Hybrid lesioned	Lesioned/ total FOG	%FOG lesioned		
1	236	22/119	18	7/30	23	43/87	49	0.001	S<u>=H</u><F (0.347)
2	394	8/226	4	2/96	2	9/72	13	0.003	S<u>=H</u><F (0.512)
3	41	3/18	17	4/16	25	7/7	100	0.001	S<u>=H</u><F (0.360)
4	258	18/135	13	2/22	9	27/101	27	0.016	S<u>=H</u><F (0.331, 3.673)
5	337	3/210	1	9/54	17	24/73	33	0.001	S<u>=H</u><F (4.392)
Mean ± SE	253 ± 60		11 ± 3		15 ± 4		44 ± 15†‡		

HU, hindlimb unloaded; H, hybrid fibers. *G values were compared to critical χ^2 value of 5.991 for $\alpha = 0.05$, and G statistics are provided in parentheses for S vs. H and H vs. F. G-statistic values ($8.23 \leq G_{table} \leq 56.5$) for the contingency tables of all 5 HU animals are significant ($P \leq 0.016$; $G_{table} \geq \chi^2_{crit,v=2} = 5.991$); null hypothesis was rejected. Heterogeneity in all 5 contingency tables is principally due to the significant F > S subset. The nonsignificant subsets of H fibers are connected with lines (solid line: $0.331 \leq G_{subset} \leq 0.512$, $0.75 \leq P \leq 0.90$; dashed line: $3.67 \leq G_{subset} \leq 4.39$, $0.10 \leq P \leq 0.15$; $G_{subset} \leq \chi^2_{crit\,0.05,v=2} = 5.991$). Animals are listed in order of damaged hybrid-fiber proportions progressing from SO-like (nonsignificant subsets, solid bars) toward FOG-like (nonsignificant subsets, dashed lines). †Significantly greater than the percentages of lesioned slow and hybrid fibers in HU muscles ($H_{stat} = 6.0897$ vs. $H_{crit,0.05,v=2} = 5.881$) ($P < 0.05$). Multiple-range test shows S < H < F ($P < 0.01$). ‡Significantly greater than average lesioned fast-fiber percentage in control rats ($P < 0.02$) ($U_{stat} = 24$ vs. $U_{crit,0.02,v=2} = 24$).

Muscle Histology

In the stimulated muscles, hematoxylin- and eosin-stained sections revealed margination of neutrophils in the intramuscular veins, an early sign of inflammation. Necrotic muscle fibers and macrophage infiltration were not observed. Sham-operated muscles showed no evidence of inflammation or myofiber necrosis.

DISCUSSION

Our initial hypothesis was that the hybrid fibers in unloaded AL muscles should be more damaged because of mismatched protein isoforms. This assertion assumed no or minimal differences in fiber-type damage between the pure FOG and pure SO fibers. What we observed is that the most damaged fiber type in 14-day HU rat AL muscles was the FOG fibers, the least damaged fiber type was the SO, and the damaged hybrid fiber proportions fell in a range between these two extremes. Because the hybrid fibers were transitional fibers, it is not surprising that they manifested damage proportions within this range. The surprising observation was that the FOG fibers were consistently, and quite dramatically, the most damaged fibers and that the SO fibers were the least damaged. It would be of interest to conduct this type of experiment on a muscle that demonstrated SO, FOG, and fast glycolytic (FG) fiber types, as the FG fiber type has long been known as extremely susceptible to eccentric contraction-induced damage (12, 13).

Sarcomere damage observed in the present study was the missing A-band type of damage (24). Missing A-band lesions are thought to form early in the sequence of sarcomere damage, which progresses to hyperstretch lesions (24). In earlier investigations in which hyperstretch lesions predominated, HU rats ambulated volitionally for 12–14 h or were subjected to an eccentric challenge then volitionally ambulated for 3–4 h (24, 26). The present study limited the AL muscles to 100 eccentric contractions over a 5-min period without voluntary reloading so that lesion progression would not be as severe. The FOG fibers consistently exhibited the largest lesions and the highest percentage of fibers damaged within all HU muscles. The percentage of lesioned fibers in control rats (~5%) was far less than that in HU rats (~15%), and the lesions observed in control rats were smaller (3.0 ± 1.0 disrupted sarcomeres/lesion) than in HU rats (7.0 ± 2.8 disrupted sarcomeres/lesion). Damage was expected to be far less severe in control AL muscles because damage susceptibility increases with unloading (18, 26, 28). Our eccentric contraction protocol was designed to induce more damage in unloaded muscles, similar to that observed with volitional reloading, using a strain that was within the physiological range of a functionally similar muscle (27).

The FOG fibers were preferentially damaged in AL muscles of all HU rats. Preferential damage of FOG fibers occurred in two of the five control rats. No fiber type was preferentially damaged in the remaining control rats. Most importantly, in no instance were slow fibers preferentially damaged in either HU or control rats. This contrasts our laboratory's previous finding in volitionally reloaded AL muscles, in which lesion damage was SO fiber specific (26). It appears that preferential SO fiber damage in unloaded AL muscles during voluntary reloading was related to the preferential activation of slow-fiber motor units during lengthening-bias movements (3, 6). Even though fast fibers are potentially more susceptible to damage, this is not manifested unless the fibers are active and stressed by

eccentric contractions (15). In agreement with others, isometric contractions were less damaging than eccentric contractions (14). In the unloaded AL, the most likely combination for damage is active contraction of a FOG fiber subjected to lengthening stress.

It might be argued that the FOG fiber-selective damage in the unloaded AL was due to uneven stresses applied to the AL, such that the rostral mixed region, in which most of the fast fibers are found, was lengthened more than the caudal SO region. This is an unlikely explanation because the present comparisons were made on contiguous SO, FOG, and hybrid fibers within single sections in the rostral region. The contiguity of fibers increased the likelihood that they experienced the same degree of lengthening. Whether individual fiber types activated at 100 Hz generated different tensions during lengthening could not be assessed in our whole muscle preparations. The greater susceptibility of fast fibers in situ is consistent with isolated, single muscle fiber studies by MacPherson et al. (14), in which fast fibers were more susceptible to damage than slow fibers. It is unknown whether the fast fibers examined by MacPherson et al. were FOG or FG fibers (14).

Could differential muscle fiber fatigue explain the greater damage of FOG fibers in unloaded muscles? Ingalls et al. (8) suggested that excitation-contraction failure explains force decrement in muscles subjected in situ to lengthening contractions. The SO and FOG fibers in the AL are both fatigue resistant (5). However, if the FOG fibers are more fatigue susceptible and drop out before SO fibers during tetany, then FOG fibers would cease generating active tension and be less prone to lesions by active lengthening, unless fatigue results in rigor, as postulated by Lieber et al. (13). The SO fibers should be the last to fatigue and should experience the longest duration of active lengthening. Differential fatigue would bias the results toward SO fiber damage. The lack of sarcomere damage in the isometrically contracted muscles, which exhibited fatigue profiles similar to those of the eccentrically contracting muscles, indicates that fatigue, by itself, is not damaging. A lack of correlation between fatigue and fiber damage was observed previously (14). Thus active lengthening during eccentric contraction, and not fatigue, damages fibers. When unloaded SO and FOG fibers are simultaneously eccentrically loaded, FOG fibers prove more susceptible to damage.

Why are unloaded FOG fibers more damage susceptible? Reports of structural differences between normal slow and fast fibers suggest higher susceptibility of fast fibers to injury (4, 7). Fast fibers appear to have narrower Z bands compared with slow fibers (4), which reflect the lower amounts of thick and thin filament tethering proteins such as titin and α-actinin (31). The relative weakness results from greater load per protein and weaker connections between sarcomeres (29, 30). Structural integrity may depend on protein isoform as well as amount. Horowits (7) reported that fast fibers express a lower molecular weight, less elastic titin isoform than slow fibers. Less compliant titin may transfer greater stress in fast fibers during lengthening. We hypothesized that hybrid fibers with admixtures of muscle protein isoforms are the most damage susceptible, but this was not substantiated in the present study. Acquisition of fast-fiber properties during unloading atrophy was expected to increase susceptibility. A threshold amount of fast properties may have to be reached before hybrid fibers manifest greater damage susceptibility than slow fibers (16). Physiological studies revealed that hybrid fibers showed no significant increase in velocity until at least 30% of the myosin was fast (16). In our study, the content of fast myosin in the hybrid fibers may have been less than 30%. Analysis of fast myosin content in hybrid fibers by immunostaining is qualitative. The issue of absolute fast myosin content and the presence of other fast muscle isoforms in hybrid fibers could not be addressed in the present investigation because of incompatible tissue processing requirements (anatomic vs. biochemical).

The present results indicate that HU rendered SO and FOG fibers in the AL more susceptible to damage than normal. Examination of eccentric contraction-induced injury in normal and 5-wk unloaded human quadriceps femoris muscles using magnetic resonance imaging revealed that chronic unloading increased vulnerability to damage (18). Factors contributing to increased damage susceptibility are unknown, but atrophy-induced loss of myofilaments may be involved. Riley et al. (19) reported a disproportionate loss of actin thin filaments with no decrease in specific tension in human soleus muscles after 17 days of unloading (19, 30). Fewer thin filaments carrying greater loads increases the likelihood of structural failure (19, 30). Warren et al. (28) reported that, in mice, HU increases the damage susceptibility of slow and fast muscles, but thin filament concentration has not been examined in rodents.

When all fibers in the unloaded rat AL muscle are active and subjected to lengthening stress, FOG fibers emerge as the most damage susceptible fiber type. These results indicate that selective damage of SO fibers in unloaded AL muscles, seen under conditions of voluntary movement, is explained by recruitment of SO fibers and relative inactivity of FOG fibers (26). Whether the FOG fiber vulnerability is inherent to the fast-fiber phenotype per se or is the consequence of a relatively lower contractile work load history has to be examined in a temporal study because unloading invokes expression of fast-fiber proteins (26, 28). HU increases damage susceptibility of both SO and FOG fibers, but FOG fibers are the most damaged fiber type in unloaded muscle.

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