Differential Response of Muscle Fibers and Interfiber Area to Increased and Decreased Neuromuscular Activity Levels

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Abstract

The purpose of the present study was to determine the relationship between the adaptations in muscle fiber size and the connective tissue content (interfiber area) in atrophying and hypertrophying skeletal muscles. Hypertrophy was induced in the cat plantaris muscle via functional overload (FO,12 weeks) induced via the removal of its major synergists. Atrophy was induced in the cat soleus muscle via a complete low-thoracic spinal cord transection (ST, 26 weeks) or spinal cord isolation of the lumbar region of the spinal cord, i.e. complete cord transections at a low thoracic and a high sacral level and bilateral deafferentation between the two transection sites (SI, 26 weeks). In addition, atrophy was induced in the rat soleus and tibialis anterior muscles via spaceflight (2 weeks) or hindlimb unloading (HU, 2 weeks). Fresh frozen tissue cross sections (20 μm thick) were stained for concanavalin A and muscle fiber area, interfiber area per fiber and the ratio of muscle fiber area : interfiber area were determined for representative regions of each muscle. FO resulted in a 133 and 139% increase in fiber area and interfiber area, respectively. In contrast, ST and SI resulted in 32 and 72% decreases in fiber area, whereas the interfiber area was unchanged. Thus, the muscle fiber area : interfiber area ratio was decreased by 37 and 69% in the soleus muscle of the ST and SI cats, respectively. For the rat soleus, there was a 39 and 28% decrease in fiber area, a 20 and 23% decrease in interfiber area, and a 24 and 10% decrease in muscle fiber area : interfiber area ratio in spaceflight and HU rats, respectively. Combined these data indicate that the relative non-muscle:muscle tissue content is maintained in hypertrophied muscles, whereas there is a disproportional loss of muscle tissue compared to non-muscle tissue in atrophied muscles. Based on these adaptations in the connective tissue and muscle tissue elements, the strain per unit of active force would decrease in atrophied muscle and remain at control levels in hypertrophied muscle.

Key words: connective tissue, fiber cross-sectional area, functional overload, interfiber area, spinal cord isolation, spinal cord transection.

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Adaptations in the contractile elements of a muscle in response to chronic decreases or increases in neuromuscular activity, i.e. activation and/or loading, are well defined (reviewed in [5, 23, 25]). The effects of alterations in muscle usage on the connective tissue matrix, however, are less well defined. For example, immobilization in a shortened position induces muscle fiber atrophy and a higher concentration of collagen, a major constituent of connective tissue [7, 12, 16, 36]. It is unclear, however, if the total content of connective tissue in the muscle is changed [7, 36]. Since immobilized muscles remain electrically active, a confounding factor is that some unknown amount of active isometric force is likely to be generated in this experimental model of atrophy [6]. In addition, an alteration in the interfiber matrix and fiber cross-sectional area (CSA) may occur in immobilized muscle due to the fact that only minimal length changes in the muscle-tendon complex are permitted.

The total daily amount of EMG activity in the cat soleus is reduced significantly following spinal cord transection (ST) at a low thoracic level [1]. Following spinal cord isolation (SI) in cats, i.e., ST at a low thoracic and a high sacral level plus bilateral dorsal rhizotomy, the muscles innervated by the motoneurons in the isolated segment of the spinal cord are nearly inactive [24]. Based on alkaline ATPase staining, Lieber et al. [17] reported an increase in the fraction of interfiber area in muscle cross-sections in both the soleus (increased from 12 to 20%) and the extensor digitorum longus (increased from 10 to 15%) of rats spinalized for one year.

The extent to which this percent increase in interfiber area was due to either muscle fiber atrophy or to an absolute change in interfiber space was not determined. Functional overload (FO), i.e., mechanically overloading a muscle by removing its synergists, induced hypertrophy in the rat plantaris and resulted in an increase in total, but no change in the concentration of, collagen measured from muscle homogenates [32].

The present study was designed to determine the extent that the tissue bed within which the contractile and related elements of muscle fibers lie atrophies when the activation level of the muscle is decreased (ST, spaceflight, hindlimb unloaded), eliminated (SI) or hypertrophied when the activation and loading levels are increased (FO). The relative adjustments of the contractile vs. the non-contractile elements of the muscle-tendon unit in models in which the contractile elements are known to expand or shrink have several potentially important physiological consequences. For example, it seems inevitable that the stress-strain characteristics of the muscle-tendon unit [10, 21] must be affected by atrophy and hypertrophy if the proportions of contractile and non-contractile elements change.

In addition to the issue of strain, the present data will provide insight into how fluid is distributed in atrophied and hypertrophied muscles. Although these data cannot be delimited to the absolute or relative contributions of interfiber space compared to the absolute or relative contributions of connective tissue, some insight is gained in the extent to which interstitial space may be altered in response to muscle atrophy or hypertrophy. For example, following 12.5-days of spaceflight the soleus muscle atrophied by ~15%, whereas the muscle fibers atrophied by ~40% [20]. This edematous effect raises questions about the interpretation of measures such as enzyme activities and fiber cross-sectional areas as well as simple contractile mechanics of whole muscle [9, 31]. These data clearly indicate that changes in the combination of space occupied by connective tissue and interstitial fluid and non-muscle fiber cells within muscles induced to atrophy or hypertrophy by reducing or elevating activity and load on the muscle are not paralleled by changes in the cross-sectional area of muscle fibers.

Materials and Methods

Functional overloaded (FO) cats

Twelve adult female cats (body weights, 2.6-3.9 kg) were divided equally into control and FO groups. The plantaris muscle was overloaded by removal of its major synergists as described by Chalmers and co-workers [3]. The cats were initially given an injection of atropine sulfate (0.10 mg/kg, i.p.) and sedated with a mixture of ketamine (13 mg/kg, i.m) and acepromazine (0.01 mg/kg, i.m.). The cats then were anaesthetized with sodium pentobarbital (35 mg/kg, i.v.) to suppress withdrawal and eye-blink responses.

Under aseptic conditions, an incision was made through the skin and fascia to expose the dorsal region of both hindlimbs. The blood supply to the soleus and medial gastrocnemius muscles were ligated, and the muscles were excised completely from both legs. The lateral gastrocnemius also was removed, except for a small fleshy portion at its proximal end where it attaches firmly to the plantaris, in order to keep the plantaris blood supply intact. Care was taken to avoid injury to the plantaris and its nerve and blood supply.

By 4 weeks post-surgery all FO cats had recovered digitigrade locomotion and were exercised daily by walking around a room for 15 min/day, 6 days/week. When the cats were able to easily tolerate this workload the exercise duration was gradually increased up to 25 min/day and the intensity increased by eliciting highly active running and jumping play activity by throwing table tennis balls around the room in which the cats played. Twelve weeks following the initial surgery the left plantaris muscle from each of the FO and control cats were removed, cleaned of fat and connective tissue and wet weighed. A 15-mm block from the mid-belly of each muscle was removed, cut into blocks approximately 1 cm long, quickly frozen in isopentane cooled by liquid nitrogen and stored at -70^{O}C until processed.

Spinal cord isolated (SI) cats

Seven female adult cats (body weights ranging between 2.3-3.8 kg) were deeply anesthetized as described above. The lumbar portion of the spinal cord was isolated as described by Pierotti et al. [24]. Briefly, under sterile conditions a skin incision was made from vertebral level T11 to S3, the fascia incised and the paraspinal muscles retracted. All dorsal spinous processes were removed from T12 to S2 and a narrow midline laminectomy (~2 mm wide) from T12 to S1 was made to expose the spinal cord. A more complete laminectomy was performed at about T12-T13 and again at L1-L2 and the spinal cord was completely transected at these sites. All dorsal roots then were severed bilaterally within the dura between the two transection sites. Post-surgical care followed the procedures described by Roy et al. [26]. The SI cats were maintained for 6 months, with the right leg of each cat receiving passive cyclic oscillations mimicking a step cycle for 30 min/day, 5 days/week during the last 5 months [27]. Based on chronic electromyographic recordings (continuous 48 hr-periods of recordings), this surgical procedure has been verified to produce a near silencing of the electrical activity in muscles in the lower limb [24]. Following a terminal in situ muscle physiological experiment, the soleus muscles were removed and prepared for histochemical analyses as described above.

Spinal cord transected (ST) cats

Eight adult female cats (body weights ranging between 2.8-3.5 kg) were deeply anesthetized as described as above. A complete ST was performed as de-

scribed by Roy et al. [26, 28]. Briefly, under sterile conditions a laminectomy and a complete spinal cord transection were performed at the T12-T13 vertebral level. Post-surgical care followed the procedures described by Roy et al. [26]. The cats were maintained for 6 months. Four ST cats were untreated and 4 ST cats were trained to stand while supporting the hindquarters 30 min/day, 5 days/week for the last 5 months of the 6 month experimental periods [27]. Following a terminal in situ muscle physiological experiment, the soleus muscles of each ST cat were removed and prepared for histochemical analyses as described above.

Control group for spinal cats

The soleus muscles from 5 female control cats (body weights ranging between 2.2-4.0 kg) were removed and prepared for histochemical analyses as described above.

Spaceflight and hind limb unloaded (HU) rats

Three groups of Czechoslovakian Wistar (SPF) male rats (n=5/group) were studied. Ground-based rats maintained under conditions, i.e., cage size, temperature, lighting, and food and water availability for the duration of the mission similar to that of the flight rats, served as synchronous controls (see [33] for details). A second group was exposed to a 14-day spaceflight (Cosmos 2044). A third group of rats were HU for 14 days. Mean initial body weights were 307, 321 and 298 g for the control, flight and HU groups, respectively. The flight rats were killed between 8-12 hr after returning to 1G. The control and HU rats were killed on a similar time schedule. At euthanasia, the control, flight and HU rats were 127, 123 and 131 days of age and had a mean body weight of 343, 338 and 339 g, respectively. At the terminal experiment, the left soleus and tibialis anterior (TA) muscles were removed and prepared for histochemical analyses as described above.

All procedures followed the Guidelines published in the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Use Committee at UCLA.

Tissue processing and analysis

Twenty-micron-thick cross sections were cut from each muscle in a cryostat at –20°C and adhered to cover slips. Following 1 hr of air drying, each section was incubated in a solution of fluorescein-conjugated concanavalin A (Con A) (Vector Laboratories, Burlingame, CA) and phosphate buffered saline (PBS) (1:100) for 1 hr. Con A binds specifically to the mannose or glucose subunit of glycoproteins or glycolipids and is thought to stain all extracellular material [19, 22]. Following three 5 min rinses with PBS, each cover slip was slide mounted with PBS and sealed with nail polish. All samples for a single muscle (i.e., the control and FO plantaris, the control, SI and ST soleus or control spaceflight and HU soleus or TA) were processed as a group on any particular day. Representative areas in the cross section of each muscle were digitized using an image processing system (PSICOM 327, Perceptive Systems Inc., Houston Texas). Tissue illumination and digitization procedures were maintained unchanged during the digitization of all of the control and experimental tissues for a single muscle. The mid-region of cat plantaris muscle shows a gradation of fast and slow fiber types across the muscle sections. Thus, 2 pictures from the deep region of the muscle, i.e., an area close to the thickened portion of the epimysium which enlarged distally to form the tendinous insertion of the muscle, and 2 pictures on the opposite (i.e., superficial) edge of the muscle were digitized. Similarly, 2-3 pictures were digitized from both the deep (near the bone) and superficial away from the bone regions of the rat TA. Because there is little difference in the distribution of fiber types across the soleus muscle, two pictures were digitized in the central region of each soleus muscle. The epimysium was not included in the regions analyzed, but no attempt was made to selectively include or exclude the perimysium.

Within each picture the number of bright interfiber Con A fluorescein positive (light) and dim fluorescein negative intrafiber (dark) pixels were determined. A cutoff between the light and dark pixels was determined by identifying the lightest pixel value within the cytoplasm of the control muscle fibers. This cutoff value was very similar across all samples for all muscles. Thus, a single value was used for all analyses. The number of fibers in each picture was determined by summing the number of fibers completely included in the picture plus half the number of fibers which were only partially included. From these measures, the percentage of interfiber area (light pixels) and muscle fiber area (dark pixels), the ratio of muscle fiber area to interfiber area, the mean fiber area and the amount of interfiber area per fiber were determined for each picture. The results from the multiple pictures taken from any muscle region were averaged within each animal. Table 1 shows a sample of how data obtained from one digitized picture was organized.

Statistical analysis

For the cat data: The ratio of the muscle area to interfiber area, fiber area and the interfiber area per fiber of the superficial and deep regions of the plantaris from the control and FO groups were compared using a two-way nested ANOVA. Post-hoc contrasts utilizing least-squared means analyses were used to determine individual group differences. For the soleus, a one-way nested ANOVA was used for overall group comparisons. For both tests, cats were nested within experiment, with the number of cats determining the degrees of freedom for the test. The level of significance was set at $P < 0.05$.

For the rat data: A one-way nested analysis of variance was used for overall group comparisons using a significance level of $P < 0.05$. The nesting variable was rats and the grouping variable was the experimental procedure. The Bonferoni adjustment was used to determine the differences between the three groups using

Table 1. Sample of data collected from one digitized picture of a fluorescein-conjugated concanavalin A plantaris muscle tissue cross-section. Each pixel equals 0.659 μm^2.

optical density used to divide light and dark pixels	76
total number of dark pixels in the image (i.e., optical density <= 76)	156741
total number of light pixels in the image (i.e., optical density > 76)	33330
number of fibers in the image	17.5
number of dark pixels per fiber	3407
number of light pixels per fiber	725
ratio of dark pixels/light pixels	4.7
percentage of dark pixels in the image	82
percentage of light pixels in the image	18

an adjusted significance level of $P < 0.017$ (.05/3). Means and standard errors of the mean are reported in the Tables and text.

The relationship between muscle fiber area and % changes in interfiber area per muscle was determined using linear regression.

Results

Adaptations in the plantaris muscles of FO cats

Control and FO cats had similar body weights (3.2 ± 0.2 vs. 3.4 ± 0.4 kg, mean ± SD), but the mean plantaris muscle mass of the FO cats was more than twice that of the control cats (10.6 ± 1.5 vs. 4.5 ± 0.5 g, $P < 0.01$). In FO muscles, the mean fiber area and associated interfiber area were increased proportionally, i.e. 233 and 239%, respectively, compared to control values (Table 2). Since interfiber area per fiber increased proportionately with fiber size, the ratio of muscle fiber area to interfiber area was unchanged in the FO plantaris. In addition, there was no change in the percentage of area in the muscle cross-section occupied by muscle fibers and interfiber area, i.e., in both control and FO muscles 82% of muscle cross section was muscle fiber area and 18% was interfiber area.

Adaptations in the soleus muscles of ST and SI cats

There were no significant differences (student *t*-tests, $P > 0.05$) between the ST cats receiving or not receiving daily standing weight-bearing training, and no difference between the oscillated and non-oscillated legs of the SI cats. Thus, these data within each group were combined. In response to reduced neuromuscular activity, there was a reduction in fiber area in both the ST (32%) and SI (72%) soleus muscles with the decrease being significantly different from control for the SI cats (Table 3). In contrast, the mean interfiber area was maintained near the control level in both the ST and SI groups. Due to the reduction in fiber area and no change in interfiber area per fiber in the ST and SI soleus muscles, the ratios of muscle fiber area to interfiber area were significantly decreased. In addition, there was an increase in the percentage of the muscle cross section occupied by interfiber area, i.e., 17, 24 and 41% in the control, ST and SI, respectively.

Adaptations in the soleus and TA of spaceflight and HU rats

Compared to control values, the mean fiber area of the soleus was significantly smaller in the spaceflight (39%) and HU (28%) groups (Table 4). There also was a tendency ($P > 0.05$) for the interfiber area per fiber to be smaller in the soleus of the spaceflight (19%) and HU (23%) rats. Consequently, the muscle fiber area : interfiber area ratios also showed a tendency ($P > 0.05$) for a decrease, and the percentage of the muscle cross section occupied by muscle tissue was similar in the three groups, i.e. 78, 73 and 77% for the control, spaceflight and HU groups, respectively.

There were no significant effects of either spaceflight or HU on any parameter in either portion of the TA muscles (Table 5).

Table 2. Fiber area, interfiber area per fiber, ratio of muscle fiber area to interfiber area and % fiber area per muscle of control and functionally overloaded (FO) plantaris muscles.

	Control	FO	% change
Fiber area (μm^2)	2592 ± 386	6028 ± 1725*	+133
Interfiber area per fiber (μm^2)	561 ± 244	1342 ± 465*	+139
Muscle fiber area / interfiber area	5.1 ± 1.2	4.9 ± 1.6	-4
Fiber area per muscle (%)	82	82	NC

Values are means ± SD. NC, no change.
* Significantly different from control, $p < 0.05$.

Table 3. Fiber size, interfiber area per fiber and ratio of muscle fiber area to interfiber area of control and spinal cord transected (ST) and spinal cord isolated (SI) soleus muscles.

	Control	ST	% change	SI	% change
Fiber size (μm^2)	3449 ± 2081	2344 ± 776	-32	963 ± 537*	-72
Interfiber area per fiber (μm^2)	714 ± 519	727 ± 132	+2	677 ± 257	-5
Muscle fiber area / interfiber area	5.2 ± 1.2	3.3 ± 1.0*	-37	1.6 ± 0.7*$^+$	-69
Fiber area per muscle (%)	83	76	-8	59	-29

Values are means ± SD.
* Significantly different from control, $p < 0.05$.
$^+$ Significantly different from ST, $p < 0.05$.

Table 4. Fiber size, interfiber area per fiber and ratio of fiber area to interfiber area of control, spaceflight and hind limb unloaded (HU) soleus muscles.

	Control	Spaceflight	% change	HU	% change
Fiber size (μm^2)	3061± 581	1853± 225*	-39	2204± 276*	-28
Interfiber area per fiber (μm^2)	857± 269	690± 128	-19.5	659± 54	-23
Muscle fiber area / interfiber area	3.0± 0.6	2.9± 0.6	-24	3.4± 0.4	-10
Fiber area per muscle (%)	78	73	-6	77	-1

Values are mean ± SD.
* Significantly different from control, $p < 0.05$.

Table 5. Fiber size, interfiber area per fiber and ratio of fiber area to interfiber area of control, spaceflight and hind limb unloaded (HU) TA muscles.

	Control		Spaceflight				HU			
	Superficial	Deep	Superficial	% change	Deep	% change	Superficial	% change	Deep	% change
Fiber size (μm^2)	3625±486	2819±776	3323±234	-8	2503±195	-11	3675±867	+1	2666±269	-5
Interfiber area per fiber (μm^2)	789±301	716±278	831±85	+5	621±133	-13	765±202	-3	613±41	-14
Muscle fiber area / interfiber area	5.1±1.4	4.2±0.9	4.2±0.7	-18	4.1±0.9	-3	5.0±0.8	-2	4.4±0.4	+3
Fiber area per muscle (%)	83	80	80	-4	80	NC	83	NC	81	+1

Values are mean ± SD.

Relationship between muscle fiber area and interfiber area across groups

There was a close interrelationship (r = 0.97) between muscle fiber area and interfiber area in atrophying muscles (Fig. 1). In contrast, this relationship was not apparent in hypertrophying muscles.

Discussion

The unique aspect of the present study is that the response of the contractile (muscle) and non-contractile (non-muscle) elements of selected muscles was determined under conditions of chronic increased (FO), decreased (ST, spaceflight and HU) and near elimination (SI) of neuromuscular activity. Morphometric analyses of muscle cross sections clearly indicate that both elements are enhanced in FO muscles, whereas the contractile element is decreased and the non-contractile element relatively unaffected in ST, SI, HU and spaceflight muscles.

Stauber and colleagues [19, 30] reported that the relative amount of connective tissue increased 28% in the rat soleus muscle following 2 weeks of HU and increased 13% in the gastrocnemius following 2 weeks of chronic centrifugation. There was significant muscle fiber atrophy in both cases. Lieber et al. [16] reported an increased fraction (from ~10 to ~20%) of connective tissue (interfiber area) in the cross sections of the dog vastus medialis and vastus lateralis, but not the rectus femoris, following 10 weeks of knee fixation. Remobilization for 4 weeks returned the connective tissue fraction, but not the fiber area, to control levels [18]. Immobilization of the ankle in a lengthened or shortened position for 1, 2 or 3 weeks in rats resulted in an increased volume and density of connective tissue in the soleus, gastrocnemius, and TA muscles [12]. In general the increase in connective tissue was

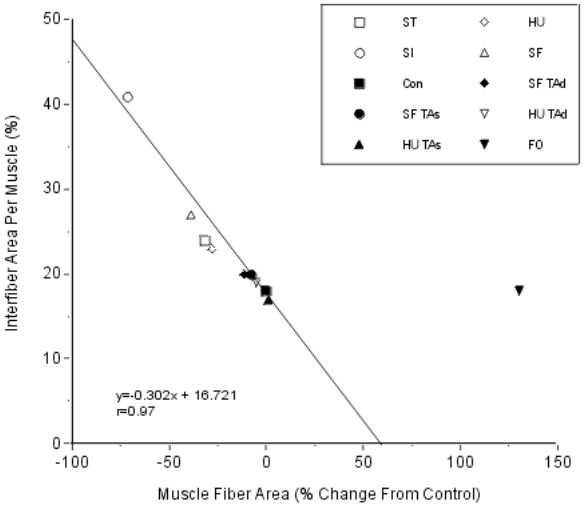

Figure 1. The relationship between the percent of interfiber area per muscle and the percent change in fiber cross-sectional area (CSA) for: Con, mean interfiber area = overall average with a range from 17 to 22%; SI, soleus from spinal cord isolated cats; ST, soleus from spinal cord transected cats; HU, soleus from hindlimb unloaded rats; SF, soleus from spaceflight rats; HU TAd and HS TAs, deep and superficial regions of the tibialis anterior of hindlimb unloaded rats; SF TAd and TAs, deep and superficial regions of the tibialis anterior of spaceflight rats; FO, plantaris of functionally overloaded cats. The coefficient of correlation (r) is 0.97, $p < 0.05$ for all points except FO.

progressive and was most evident in the soleus and least evident in the TA. There also was a significantly higher connective tissue fraction in the soleus muscle immobilized in a lengthened than shortened position. These same investigators reported similar findings following tenotomy of the Achilles tendon [11]. Kannus et al. [13] reported an increase in the percent connective tissue from 4 to 19% and in a 31% decrease in fiber size in the rat soleus immobilized in a shortened position for 3 weeks. Subsequent free cage activity for 8 weeks had little effect on the connective tissue or fiber size, but low- and high-intensity treadmill activity restored the changes towards control levels. The high-intensity activity was more effective than the low-intensity activity, suggesting that the level of loading was an important factor (see below). Following 8 weeks of immobilization or denervation of the rat gastrocnemius and soleus muscles, muscle atrophy was accompanied by an increased concentration but no change in total collagen content [7]. In the mouse soleus muscle, there was a progressive increase in the concentration of collagen (biochemically) and connective tissue (histochemically) during 4 weeks of immobilization in a shortened position [36]. The denervated rat gastrocnemius showed an increase in the relative amount of connective tissue and changes in the distribution of collagen types over a 30-day period [29]. Although all of these data suggest that the concentration of connective tissue increases in atrophied muscles, it is not clear whether the total content of connective tissue within the muscle changes.

Similar to that observed following limb immobilization or denervation, there was an increased proportion of the muscle cross-section occupied by interfiber area in the atrophied soleus muscles of ST or SI cats and spaceflight and HU rats. This increased concentration was due largely to a reduction in the muscle fiber size with no change in interfiber area. Furthermore, daily passive stretching of the SI muscles or daily standing exercise in the ST cats had a minimal effect on the fraction of interfiber area in the atrophied muscles. Thus, our results would indicate that the amount of connective tissue remains relatively stable in atrophied muscles, whether or not daily sessions of alternating strain was imposed on the muscles.

In the present study, a greater than doubling of muscle fiber size following FO resulted in a proportional increase in muscle fiber area and interfiber area in the cat plantaris. Similarly, Turto et al. [32] reported significant increases in the weight and collagen content, but not concentration, of the rat plantaris 18 days after tenotomy of the gastrocnemius muscle. However, the connective tissue response to hypertrophy may differ in muscles composed primarily of either slow or fast fibers. For example, hypertrophy of the rat soleus 3 weeks following tenotomy of the gastrocnemius and plantaris muscles resulted in an increased concentration of collagen, measured biochemically and histochemically [34]. In the hypertrophied FO cat plantaris muscles in the present study there was a significant increase in the percentage of slow fibers [3]. Muscles that play a role in maintaining posture contain a higher percentage of slow fibers and have a higher concentration of collagen than muscles comprised primarily of fast fibers (based on hydroxyproline data) (7, 12, 14, 17]. Thus, it was somewhat surprising that the plantaris muscles in the present study adapted to the condition of elevated neuromuscular activity by increasing the percentage of slow fibers, yet there was no increase in the relative interfiber area. Incrementally applied stretch of the rabbit latissimus dorsi via an implanted tissue expander results in muscle fiber hypertrophy and increased intermuscular connective tissue: the percentage of the muscle cross section occupied by connective tissue is significantly increased from 15 to 19% at 3 weeks and then returns to control levels at 6 weeks [4]. These latter data suggest that there may be a more rapid response of the connective tissue than the muscle tissue elements associated with hypertrophy.

The effects of the observed adaptations in the ratio of muscle fiber area : interfiber area, in the absence of any other changes would alter the mechanical properties of the musculo-tendinous unit. For example, Alnaqeeb et al. [2] reported an increase in the interfiber connective tissue

(and collagen) in the muscles of old rats and this was accompanied by an increase in muscle stiffness when the muscle was stimulated, particularly in the fast muscles. Interestingly, the lack of a change in stiffness of the slow soleus muscle was attributed to the fact that the soleus is a highly active muscle, even at an advanced age. The changes observed in the quantity and quality of intramuscular connective tissue associated with immobilization also result in greater passive stiffness [35] and affect the muscle's resistance to contraction-induced injury, i.e. muscles immobilized in a lengthened position have a higher connective tissue content and are less susceptible to contraction-induced injury than muscles immobilized in a shortened position and both are more resistant to injury than control muscles [15]. Increased passive stiffness has also been observed in HU rats and was thought to be due, at least in part, to changes in the connective tissue elements [8]. If activity does indeed play a role in maintaining the normal compliance of the musculo-tendinous unit, then large changes in stiffness may be expected to occur in muscles experiencing a chronic decrease or elimination of neuromuscular activity. The changes in the muscle fiber area : interfiber area do not preclude that other adaptations could occur which could affect the stiffness of the musculo-tendinous complex in either a passive or active mode. Qualitative changes in the properties of both the muscle fibers and its surrounding matrix could easily contribute to the stress-strain properties of atrophied or hypertrophied muscles.

In summary, the results indicate that 1) interfiber area increases in proportion to muscle fiber CSA in response to hypertrophy of a fast muscle; and 2) interfiber area is maintained at control levels in response to muscle fiber atrophy in both slow and fast muscles. These results suggest that when neuromuscular activity is elevated above normal levels there is a concomitant increase in the connective tissue matrix, but when neuromuscular activity levels are reduced the connective tissue matrix is maintained at control levels. These changes in the ratio of connective tissue matrix to contractile material most likely affect the passive length-tension and kinetic properties of the muscle-tendon unit.

Abbreviations

Con A, concanavalin A; CSA, cross-sectional area; FO, functional overload; HU, hind limb unloading; PBS, phosphate buffered saline; SI, spinal cord isolation; ST, spinal cord transection; TA, tibialis anterior.

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