Hindlimb unloading induces a collagen isoform shift in the soleus muscle of the rat

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Hindlimb unloading (HU) is a model for the evaluation of the effects of microgravity on tissues. In the present study, we examined the effects of 11, 14, and 28 days of HU on the mechanical and biochemical properties of the soleus muscle of male Sprague-Dawley rats. The effects of HU were compared with those of tail suspension (microgravity) for 11, 14, and 28 days. Hindlimb unloading induced a collagen isoform shift in the soleus muscle, but tail suspension did not. The proportion of collagen type I fibers decreased and the proportion of collagen type III fibers increased with HU. The total hydroxyproline content in the soleus muscle decreased relative to control (Con) at 14 and 28 days HU. However, the relative proportions of type I collagen in the antigravity plantaris muscle were unchanged with HU. The data suggest that HU-induced alterations in the ECM are involved in the passive mechanical properties of muscle. The effects of HU on the ECM and the mechanical properties of soleus muscle have been previously investigated (4, 5). In these studies, 3 wk of HU resulted in a significant decrease in the passive stiffness of the soleus muscle. However, these studies did not examine whether alterations in the ECM contributed to the mechanical changes induced by HU. Therefore, the purpose of this study was to determine whether HU alters the gene expression for type I and III collagen as well as the abundance and isoform of skeletal muscle collagen and the degree of collagen cross-linking. We hypothesized that total collagen abundance and cross-linking would decrease with HU and that HU would induce a type I-to-type III shift in collagen isoform and gene expression.

METHODS

The methods employed in this study were approved by the Texas A&M University Laboratory Animal Care Committee. The investigation conformed to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892].

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**Experimental animals.** Eight-month-old male Sprague-Dawley rats were obtained (Harlan) and housed in an environmentally controlled room maintained at 23 ± 2°C and kept on a 12:12-h light-dark cycle. The animals were given food (commercial rat chow) and water ad libitum. The rats were randomly assigned to one of four age-matched groups; control (Con; n = 11), 1-day hindlimb-unloaded (1 day HU; n = 11), 14-day hindlimb-unloaded (14 days HU; n = 13), or 28-day hindlimb-unloaded (28 days HU; n = 11) group. The hindlimbs of the HU animals were elevated to an approximate spinal angle of 40–45° from horizontal, as previously described (6, 7). Briefly, the animals were injected with pentobarbital sodium (30 mg/kg ip Nembutal, Abbott Labs) to induce light anesthesia. While anesthetized, the animal’s tail was washed and dried, and a length of breathable non-elastic adhesive tape (Curity Porous tape, Kendall) with a hook attached to the end was placed on the proximal two-thirds of the tail. The ends of the adhesive tape were further bonded to the tail with an additional adhesive (GOOP) and allowed to dry for 20 min before suspension. Daily inspection of animals’ tails was performed, checking for discoloration or tissue damage from the suspension apparatus. The hook attached to the adhesive tape was connected by a small chain to a swivel apparatus fixed at the top of the cage. Adjustments to the length of the chain were made as necessary to prevent the rat hindlimbs from touching any supportive surfaces while the forelimbs maintained contact with the cage floor. This allowed the animals free range of movement about the cage. Con animals were kept in a normal cage environment, whereas HU rats were maintained in the unloaded position. On completion of the treatment protocol, animals were anesthetized with pentobarbital sodium (60 mg/kg ip), decapitated, and the soleus and plantaris muscles were excised from both hindlimbs and weighed. Muscle sections to be used for histological analysis were placed in a PBS solution of 7% glycerol and 4% sucrose for 30 min before freezing in isopentane cooled with liquid nitrogen. Muscles from the contralateral limb were divided in half, frozen in liquid nitrogen, and stored at −80°C for measurement of hydroxyproline (HYP) and mRNA for types I and III collagen.

**HYP analysis.** HYP quantification was performed according to the method described by Bergman and Loxley (2). Briefly, muscle samples were freeze-dried for 24 h, weighed, and hydrolyzed in 6 N hydrochloric acid at 120°C for 18 h. Samples were filtered before the assay was performed. Samples were added to isopropanol, an oxidant solution consisting of chloramine and citrate buffer, and Ehrlich’s solution before overnight incubation. After incubation, absorbance of the samples was read at 558 nm and plotted against standard concentrations. Muscle collagen concentration was determined from HYP when expressed as micrograms HYP per milligrams muscle dry weight. However, total muscle collagen content was determined using the following equation

\[
\mu g \text{ HYP/whole muscle} = (\mu g \text{ HYP/g sample wet wt}) \times g \text{ whole muscle wet wt}
\]

**Collagen staining with picrosirius red.** Transverse 7-μm sections of soleus and plantaris muscles were fixed in 10% neutral buffered zinc formalin (Anatech, Battle Creek, MI) for 10 min and rehydrated for 10 min in distilled water. Collagen staining was performed using a modification of the combined methods described by Sweat et al. (29) and Dolber and Spach (9). Briefly, following a 2-min incubation in 0.2% phosphomolybdic acid, samples were stained with a solution of 0.1% picrosirius red (PSR) (Sirius Red F3B and saturated picric acid) for 90 min. Samples were rinsed 2 min in 0.01 N HCL, followed by 1-min rinses in 70% ethanol, 100% ethanol, and xylene. Samples were mounted using Cytoseal 60 (Spectronics, Westbury, NY). The tissue was viewed using polarization microscopy for identification of collagen types I and III (15, 16). Staining of tissue using the PSR technique results in a nonspecific red staining of collagen when viewed under nonpolarized light. Examination of sections under polarized light results in enhanced birefringence of collagen with type I collagen appearing orange to bright red and type III collagen appearing green (16). The red or green appearance of collagen is largely dependent on the thickness of the collagen fibers and not the molecular composition of the collagen. Therefore, when the PSR method is used, it is critical that tissue section thickness is consistent among all samples, because tissue thickness will influence the viewed color of the collagen. Collagen cross-sectional area (CSA) was analyzed using a Bioquant image analysis system (R&M Biometrics, Nashville, TN). This system consists of an Olympus BX-60 microscope with an attached Optronics DEI 470 camera interfaced with a personal computer.

Muscle collagen concentration was determined using the PSR technique according to the following equation

**Type I and III collagen (% muscle CSA)**

\[
\frac{(\text{Type I} + \text{Type III collagen CSA})}{\text{total muscle section CSA}} \times 100
\]

The composition of types I and III collagen as a percentage of total collagen was determined according to the following equations

**Type I collagen (%)**

\[
\frac{\text{Type I collagen CSA}}{(\text{Type I} + \text{Type III collagen CSA})} \times 100
\]

**Type III collagen (%)**

\[
\frac{\text{Type III collagen CSA}}{(\text{Type I} + \text{Type III collagen CSA})} \times 100
\]

**Analysis of hydroxypyridinium cross-links.** Hydroxypyridinium (HP) concentration in soleus muscle was quantified by high-performance liquid chromatography with fluorescent detection as previously described (10). Briefly, muscle samples were hydrolyzed as described above, and heptafluorobutyric acid (HFBA) was added to an aliquot to obtain a final concentration of 1%. Samples were chromatographed as described with 0.1% HFBA in the elution buffer. HP concentration was determined from a standard curve generated with purified HP (Metra Biosystems, Mountainview, CA).

**Collagen types I and III mRNA analysis.** Soleus muscle (50–100 mg) was used for measurement of types I and III collagen mRNA. Briefly, the muscles were placed in RNA Isolator (Genosys, Woodlands, TX) and homogenized in a PowerGen homogenizer. Standard chloroform/phenol extraction procedures were used to remove RNA (1). The concentration of RNA was quantified first by measuring absorbance at 260 nm. RNA samples (10 μg) were then loaded onto a 1% denaturing agarose gel and electrophoresed at 90 V for 2 h. The gel was stained with ethidium bromide and photographed under UV light to determine the integrity of the RNA. After electrophoresis, RNA was transferred overnight to Brightstar (Ambion, Austin, TX) nylon membrane by upward capillary action. After the transfer, the RNA was UV cross-linked to the membrane using a Spectrolinker UV cross-linker (Spectronics, Westbury, NY). The membrane was hybridized with 32P-labeled cDNA probes (donated by Dr. Y. Yamada from NIH, Bethesda, MD) for types I and III.
collagen. For the type I collagen probe, the 5′ and 3′ ends of the clone corresponded to residues 2096 and 4270 of mRNA for mouse c2(I) collagen chain (GenBank accession #X58251). For the type III collagen probe, the 5′ end of the clone corresponds to residues 339 of cDNA (GenBank accession #X70369) for rat α1(III) collagen. Membranes were also probed for the 18S ribosomal band with DECappedrobe template (Ambion). To quantify collagen mRNA, pixel intensity of the bands of interest was normalized to the pixel intensities of their respective 18S ribosomal band using ImageJ software (NIH).

Immunohistochemical analysis for muscle fiber types. Serial transverse 7-μm cross sections of frozen soleus and plantaris muscles were cut on a cryostat microtome. Fiber type identification was performed as described by Schiaffino et al. (28). Sections of muscle were fixed with cold AFA fixative (50 ml 37% zinc formalin + 370 ml 95% ethanol + 25 ml glacial acetic acid) for 5 min. Slides were then hydrated for 10 min in PBS before blocking. PowerBlock solution (InnoGenex #BS-1310-25) was added to the sections and incubated for 5 min at room temperature. After removal of excess blocker, primary antibodies to the myosin heavy chains, type I (BA-D5), type IIA (SC-71), and type IIB (BF-3), were added to the appropriate sections, and the slides were incubated at 4°C overnight in a humid chamber. After incubation, slides underwent a 2 × 10-min wash in PBS with gentle rotation. After being washed, a biotinylated goat anti-mouse Ig secondary antibody (InnoGenex #AS-2400-16) was added to the sections for 20 min at room temperature. Slides were washed as described above, and streptavidin alkaline phosphatase conjugate (InnoGenex #CJ-1002-86) was added to the sections and incubated for 20 min at room temperature. The conjugate was removed by washing (as in prior steps), and a solution of naphthol phosphate buffer (InnoGenex #CH-0802–04) was added to the sections and incubated until adequate color development was seen. Sections were counterstained with Mayer’s hematoxylin and mounted with Glycergel (Dako, Carpinteria, CA). Fibers containing the myosin heavy chains of interest expressed a red color following exposure to the immunohistochemical staining procedure. Serial muscle sections were also examined for IIX fibers (i.e., fibers that expressed no staining following exposure to any of the heavy chain antibodies) and hybrid fibers (i.e., fibers that expressed multiple heavy chains). All fibers in each muscle cross section were typed to determine the relative population of each fiber type.

For the quantification of fiber CSA, muscle cross sections were divided into four or five evenly spaced regions as previously described (8). Representative fascicles with fibers cut perpendicular to their long axes were chosen from each of the regions for measurement of fiber areas. Muscle fiber CSA was measured from an outer diameter tracing with the use of the Bioquant image-processing system. A minimum of five fibers of each type was measured in each of the four or five regions of the muscle cross section. Therefore, in every muscle, fiber area for each of the fiber types was measured in 25–40 fibers. Exceptions to this procedure were made when only a few fibers of a given fiber type were present in a muscle. Under this circumstance, fiber CSA was measured in all the fibers present of that type in the muscle cross section.

Data analysis. Results were analyzed using a one-way ANOVA, and post hoc analyses were performed using a Student-Newman-Keuls test. All results are means ± SE and tested at P < 0.05 level of significance.

RESULTS

Muscle mass. Chronic HU resulted in atrophy of both the soleus and plantaris muscles (Table 1). With 14 days HU, soleus and plantaris muscle masses were 34 and 24% lower than that in standing Con animals, respectively. With 28 days HU, soleus and plantaris muscle masses were 47 and 24% lower than that in Con rats, respectively.

Muscle collagen. Muscle collagen concentration as determined from HYP concentration (μg HYP/mg of dry muscle) was greater in both the soleus and plantaris muscles of 14 and 28 days HU (Table 1). Similarly, when collagen concentration (as determined by the sum CSA of types I and III collagen) was expressed as a percentage of muscle CSA, there was an increase in muscle collagen concentration with 14 and 28 days HU in the soleus (Fig. 1) and plantaris muscles (Table 1). However, when the absolute amount of HYP was expressed as micrograms HYP per whole muscle, total muscle collagen content did not change in either the soleus or plantaris muscles with HU (Table 1).

Types I and III collagen. The soleus muscle contained a greater percentage of type I collagen than plantaris muscle (Fig. 2), whereas the plantaris muscle contained a greater proportion of type III collagen (Fig. 3). With HU, the relative proportion of type I collagen decreased in soleus muscle following 14 and 28 days HU relative to that of Con, whereas no change in the

Table 1. Muscle mass and collagen composition of rat soleus and plantaris muscles in control and 1-, 14-, and 28-day HU animals

<table>
<thead>
<tr>
<th></th>
<th>Soleus</th>
<th>Plantaris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1 Day HU</td>
</tr>
<tr>
<td>Muscle mass, mg wet wt</td>
<td>253 ± 10</td>
<td>268 ± 8</td>
</tr>
<tr>
<td>Collagen concentration</td>
<td>HYP, μg HYP/mg muscle dry wt</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Type I + III collagen, % muscle CSA</td>
<td>5 ± 0.4</td>
<td>4 ± 0.5</td>
</tr>
<tr>
<td>Collagen content</td>
<td>HYP, μg HYP/whole muscle</td>
<td>621 ± 52</td>
</tr>
<tr>
<td>HP cross-link expression, mol HP/mol collagen</td>
<td>0.29 ± 0.04</td>
<td>0.24 ± 0.02</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 11 for each group except the 14-day hindlimb unloaded (HU) group, where n = 13. *Group mean is different from control and 1-day HU means (P < 0.01). †Group mean is different from 14-day HU mean (P < 0.05). HYP, hydroxyproline; HP, hydroxyprolyrindium; CSA, cross-sectional area.
proportion of type I collagen occurred in the plantaris muscle (Fig. 2). Conversely, the relative proportion of type III collagen in the soleus muscle of 14- and 28-day HU animals increased compared with Con with no change in the proportion of type III collagen in the plantaris muscle (Fig. 3).

**HP cross-link expression.** There was no difference in the expression of HP cross-linking in the soleus muscle among groups. (Table 1).

**Types I and III collagen mRNA.** HU had no apparent effect on the expression of mRNA for types I and III collagen in the soleus muscle (Fig. 4).

**Myofiber phenotype.** HU for 14 and 28 days resulted in a decrease in the proportion of type I myofibers in...
the soleus muscle (Table 2). Correspondingly, there was an increase in the number of hybrid myofibers coexpressing the types I and IIB myosin heavy chains in the soleus muscle of 14- and 28-day HU rats. Atrophy of types I and IIA fibers, as indicated by decreases in fiber CSA, occurred in the soleus muscle of 14- and 28-day HU animals (Table 2). In the plantaris muscle, HU did not alter the myofiber composition, but atrophy of all myofiber populations occurred following 14 and 28 days HU (Table 2).

**DISCUSSION**

Previous work has demonstrated that the stiffness of slow-twitch soleus muscle decreases with HU (4, 5). The primary purpose of this study was to determine whether this alteration in muscle stiffness may be related to a remodeling of the soleus muscle with respect to collagen abundance, isoform, and cross-linking. There was no change in the absolute amount of collagen in soleus or plantaris muscles with unloading (Table 1), as indicated by no change in the amount of HYP per muscle, or an alteration in HP cross-link expression in the soleus muscle (Table 1). However, with 14 and 28 days HU, there was a decrease in the proportion of type I collagen (Fig. 2) and an increase in the proportion of type III collagen (Fig. 3) in the soleus muscle but not the plantaris muscle. These alterations in the soleus muscle collagen composition did not appear to be the result of changes in collagen types I or III mRNA expression (Fig. 4).

In the previous work reporting that 3 wk of HU decreases the passive stiffness of rat soleus muscle (4, 5), the authors suggested several contributing factors that may result in decreased muscle stiffness. These included alterations in the series elastic component (SEC) of the muscle and alterations in the active structures of the SEC, such as the actin/myosin cross bridges, which have been implicated as a contributing factor to decreased stiffness. In addition, it has been suggested that a decline in the number of actin/myosin cross bridges per muscle fiber occurs following HU (23). Changes in the passive tendinous structures of the SEC have also been suggested as a potential factor contributing to increased muscle compliance. This suggestion is supported by the work of Nakagawa and colleagues (24), who report a decreased diameter of the Achilles tendon following HU. Additionally, decreases in the collagen content of the patellar tendon have been reported following HU (31). This decrease in tendon collagen content was attributed to a decrease in localized tendon strain that accompanied HU.

On the basis of the reports of decreases in collagen content of tendon with HU, we hypothesized that decreased loading would similarly result in a decreased expression of muscle collagen. The results of the present study do not support this hypothesis. In fact,

**Table 2. Composition and CSA of myofibers from rat soleus and plantaris muscles of control and 1-, 14-, and 28-day HU animals**

<table>
<thead>
<tr>
<th>Fiber composition, %</th>
<th>Control</th>
<th>1 Day HU</th>
<th>14 Day HU</th>
<th>28 Day HU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>96 ± 2</td>
<td>96 ± 1</td>
<td>86 ± 1*</td>
<td>83 ± 1*</td>
</tr>
<tr>
<td>Type IIA</td>
<td>3 ± 1</td>
<td>4 ± 2</td>
<td>5 ± 2</td>
<td>0.00</td>
</tr>
<tr>
<td>Type IIX</td>
<td>0.00</td>
<td>0.00</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Type IIB</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>0.00</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Type I/II hybrid</td>
<td>0.00</td>
<td>0.00</td>
<td>8 ± 2*</td>
<td>14 ± 3*†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fiber CSA, μm²</th>
<th>Control</th>
<th>1 Day HU</th>
<th>14 Day HU</th>
<th>28 Day HU</th>
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<tbody>
<tr>
<td>Type I</td>
<td>2,503 ± 80</td>
<td>2,820 ± 171</td>
<td>1,426 ± 134*</td>
<td>918 ± 61†</td>
</tr>
<tr>
<td>Type IIA</td>
<td>2,574 ± 199</td>
<td>2,720 ± 78</td>
<td>1,156 ± 102*</td>
<td>—</td>
</tr>
<tr>
<td>Type IIX</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Type IIB</td>
<td>1,544 ± 263</td>
<td>2,013</td>
<td>—</td>
<td>757 ± 65</td>
</tr>
<tr>
<td>Type I/II hybrid</td>
<td>—</td>
<td>—</td>
<td>1,947 ± 196</td>
<td>1,572 ± 172</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 11 for each group except the 14-day HU group, where n = 13. Fiber CSA for each fiber type was determined in ~25–40 fibers per muscle, with several exceptions (see METHODS for details). *Group mean is different from control and 1-day HU means (P < 0.01). †Group mean is different from 14-day HU mean (P < 0.05).
collagen concentration increases (Fig. 1) when expressed both as a function of muscle dry weight or muscle CSA (Table 1). This increase in muscle collagen is primarily due to the muscle atrophy induced by HU. Muscle fiber atrophy is evident from the decrease in muscle mass (Table 1) and myofiber diameter (Fig. 1 and Table 2). However, when collagen is expressed as an absolute amount of collagen per whole muscle, there is no change in intramuscular collagen content with HU (Table 1). Therefore, a decrease in intramuscular collagen does not appear to be a mechanism that contributes to decreased muscle stiffness with HU.

In addition to changes in collagen abundance, alterations in the degree of collagen cross-linking would have a profound effect on the mechanical properties of skeletal muscle. For example, Kovanen and colleagues (19) have shown that when cross-link formation is reduced by feeding rats the lysyl oxidase inhibitor β-aminopropionitrile, there is a corresponding decrease in soleus muscle stiffness. Therefore, decreases in the expression of the HP cross-link in soleus muscle with HU would be consistent with the decrease in passive stiffness. However, HP cross-linking does not change in the soleus muscle following HU (Table 1) and thus cannot account for the change in muscle stiffness.

Although decreases in collagen abundance and cross-linking do not explain the decrease in muscle stiffness associated with HU, changes in the proportions of types I and III collagen are consistent with this adaptation. For example, type III collagen correlates with tissue compliance due to its ability to form thinner, more elastic fibers than type I collagen. The hypothesis that a change in collagen isoform may affect tissue stiffness is supported by the fact that fast-twitch muscle, which contains a higher relative proportion of type III collagen than slow-twitch muscle, possesses a lower passive stiffness than slow-twitch muscle (26). Thus an HU-induced increase in the proportion of type III collagen and a corresponding decrease in the proportion of type I collagen in the soleus muscle are likely contributors to the decrease in soleus muscle stiffness. On the basis of the work of Kovanen et al. (19) demonstrating that muscle collagen is the primary determinant of the muscle mechanical properties, the change in the proportion of types I and III collagen with HU would likely be of great functional significance.

The production of collagen protein is frequently regulated at the transcriptional level (25), and thus the expression of the fibrillar collagen proteins often follows the same trend as the expression of their respective mRNAs. In the present study, however, collagen mRNA levels in the unloaded soleus muscle were not different from that of control muscle. Therefore, HU-induced alterations in muscle collagen appear to be posttranscriptionally regulated. Previous work investigating immobilized rat hindlimb muscle has also shown no change in types I or III collagen mRNA expression following 1 day of immobilization (14). In the same study, type I collagen mRNA expression was decreased in soleus muscle following 3 days of immobilization, but it returned to control levels after 7 days of immobilization. Type III collagen mRNA, however, was lowered in soleus following both 3 and 7 days of immobilization. Immobilization did not result in changes in the abundance of total intramuscular collagen or the relative proportions of types I or III collagen. These results therefore support the notion that the expression of fibrillar collagen proteins does not necessarily follow the expression of the mRNA. However, it also indicates that collagen mRNA levels may have changed between 1 and 14 days of unloading. For example, there appeared to be a tendency for type I collagen mRNA to decrease and type III mRNA to increase with HU. Therefore, the possibility that mRNA expression may have been altered at some intermediary time period cannot be excluded.

In addition to HU-induced alterations within the ECM, the myofiber composition of muscle may also affect its mechanical properties. For example, inherent differences have been shown to exist between the stiffness of fibers from fast-motor units and those from slow-motor units, with slow fibers possessing the greater stiffness (26). This relation between fiber type and stiffness also appears to be preserved in muscle undergoing fiber transformation. Soleus muscles from rats having undergone vertical jump training have been reported to exhibit a lower stiffness than that from Con animals; the soleus muscles from these trained animals had a decreased expression of type I and an increased proportion of type II myofibers (27). Conversely, endurance training has been reported to induce increases in soleus muscle stiffness concomitantly with a fast-to-slow-twitch myofiber transformation (13). Thus these studies indicate that the direct relation between slow-twitch fiber composition and muscle stiffness is maintained with adaptive myofiber transformation. The slow-to-fast myofiber transformation that occurs with HU (Table 2) is also consistent with the notion that muscle stiffness corresponds to adaptive changes in muscle fiber composition.

Changes in muscle stiffness could have functional consequences on the muscle’s ability to store and release elastic energy during locomotion (30). For example, a stiffer muscle, such as the slow-twitch soleus muscle, exhibits a greater capacity to store elastic energy than a more compliant fast-twitch muscle (19). The storage and release of elastic energy, particularly during locomotion, provide a significant portion of the mechanical energy required for whole body movement. During running, elastic energy that is stored in muscle during its lengthening phase is reclaimed to reduce the amount of energy expended during the subsequent shortening muscle phase (19, 32). Therefore, the mechanical efficiency of muscle may be profoundly influenced by its passive stiffness.

Alterations in intramuscular collagen composition that diminish mechanical stiffness of muscle may ultimately lower the muscle’s fatigue resistance. The alterations in collagen isoform found in this study are consistent with previous findings that HU decreases the stiffness of the slow-twitch soleus muscle (4, 5). Furthermore, fatigability of the soleus muscle has been...
shown to increase following 15 days of HU. McDonald and colleagues (22) have shown that intense electrical stimulation of the soleus muscle following HU resulted in significantly faster rates of fatigue following 1 min of stimulation compared with muscles from Con animals.

In conclusion, a relatively large body of work exists to support the fact that varying mechanical loading patterns can induce alterations in muscle stiffness. Although several potential mechanisms exist that may alter the mechanical properties of muscle, alterations in intramuscular collagen have been shown to be a predominant determinant of these properties (19). In the present study, several morphological alterations occurred following HU that may affect the mechanical properties of muscle. Although no changes in the absolute amount of intramuscular collagen or collagen cross-linking occurred with HU, decreases in the proportion of type I collagen, increases in the proportion of type III collagen, and the slow-to-fast myofiber transformation are consistent with the decrease in soleus muscle stiffness following HU. Decrements in muscle stiffness induced by HU may also have a profound effect on the muscle’s ability to store elastic energy and thus increase the fatigability of the soleus muscle during locomotion.

Perspectives

The present study demonstrates that the unloading of a postural muscle results in a collagen isof orm shift and myofiber transformation that favor a decrease in muscle stiffness. Such decrements in stiffness will undoubtedly adversely affect the ability of muscle to mechanically store elastic energy and therefore contribute to the increased fatigability of muscles during exercise. Although the present studies were performed in a rat model, the results may have broader implications and provide insight into the mechanisms of increased muscle fatigability for other conditions involving muscle endurance training.

REFERENCES