Gosselin, Luc E., Christopher Adams, Tiffany A. Cotter, Richard J. McCormick, and D. Paul Thomas. Effect of exercise training on passive stiffness in locomotor skeletal muscle: role of extracellular matrix. J. Appl. Physiol. 85(3): 1011–1016, 1998.—The purpose of this study was to evaluate the effect of endurance exercise training on both locomotor skeletal muscle collagen characteristics and passive stiffness properties in the young adult and old rat. Young (3-mo-old) and senescent (23-mo-old) male Fischer 344 rats were randomly assigned to either a control or exercise training group (young control [YC], old control [OC], young trained [YT], old trained [OT]). Exercise training consisted of treadmill running at ~70% of maximal oxygen consumption (45 min/day, 5 days/wk, for 10 wk). Passive stiffness (stress/strain) of the soleus (Sol) muscle from all four groups was subsequently measured in vitro at 26°C. Stiffness was significantly greater for Sol muscles in OC rats compared with YC rats, but in OT rats exercise training resulted in muscles with stiffness characteristics not different from those in YC rats. Sol muscle collagen concentration and the level of the nonreducible collagen cross-link hydroxylysylpyridinoline (HP) significantly increased from young adulthood to senescence. Although training had no effect on Sol muscle collagen concentration in either age group, it resulted in a significant reduction in the level of Sol muscle HP in OT rats. In contrast, exercise had no effect on HP in the YT animals. These findings indicate that 10 wk of endurance exercise significantly alter the passive viscoelastic properties of Sol muscle in old but not in young adult rats. The coincidental reduction in the principal collagen cross-link HP also observed in response to training in OT muscle highlights the potential role of collagen in influencing passive muscle viscoelastic properties.

COLLAGEN, the major structural constituent of the interstitial space (14), provides a scaffold for maintaining muscle-tendon integrity and is involved in the transmission of muscular forces (3). Collagen is architecturally assembled such that it affords very little resting force in muscle at or below optimal length \( L_o \). However, as the muscle is stretched beyond \( L_o \), the elastic properties of collagen are responsible in part for the passive force developed (3). Similar to other tissues, the load-deformation curve of skeletal muscle is approximately exponential (9). Several factors related to collagen may contribute to the passive viscoelastic properties of skeletal muscle, including the amount of collagen, its phenotypic distribution (2, 16), the extent of collagen cross-linking, and the architectural organization of the collagen fibrils (13, 16).

The maturation of collagen, an essential posttranslational process involving the formation of nonreducible cross-links, has been described, but factors regulating the process are poorly understood (6, 22). The rate-limiting step involves the oxidation of lysine and hydroxylysine residues by the enzyme lysyl oxidase, and the major end result in skeletal muscle is the formation of the acid-stable collagen cross-link HP (6, 22). The maturation of collagen alters the mechanical and chemical properties of the protein, leading to increased stability and tensile strength (6, 29), decreased solubility (23), and enhanced resistance to some proteases (4).

Aging is associated with significant changes in the connective tissue compartment of skeletal muscle. An increase in both concentration of collagen and extent of nonreducible cross-linking occurs with aging in both skeletal muscle (8, 33) and heart (28). The age-related changes are thought to be the result of decreased collagen turnover rates. The biochemical changes of collagen have been correlated with changes in muscle stiffness, i.e., stress/strain, such that stiffness increases in muscles of old animals (8, 10).

Exercise training is associated with increased prolyl-4-hydroxylase (PH) activity in skeletal muscle and heart without an increase in collagen concentration (11, 27), suggesting a higher collagen turnover rate. As a result of the increased rate of both collagen deposition and degradation, collagen cross-linking decreases in both skeletal muscle and heart from old trained animals (28, 33). It has been hypothesized that a reduction in collagen cross-linking in skeletal and cardiac muscle might contribute to decreased passive stiffness (28, 33); however, this hypothesis has not been tested. The purpose of this study was to determine the effects of endurance exercise training on locomotor muscle passive stiffness in both young and old rats. Our hypothesis was that exercise training of muscle in old animals, with its resulting decrease in collagen cross-linking, would similarly attenuate the age-associated increase in passive muscle stiffness.

METHODS

Animals. Young (age 3 mo) and old (age 23 mo) male Fischer 344 rats were obtained from the National Institute on Aging (Bethesda, MD) and housed according to State University of New York at Buffalo Institutional Animal Care and Use Committee guidelines. Rats were maintained on a 12:12-h light-dark cycle and were provided with water and commercial rat chow ad libitum.

Training protocol. The young and old rats were randomly assigned to either a sedentary control (young control [YC],
and placed in cooled (4°C) Krebs solution (2.5 mM Ca²⁺). Adult (age 6 mo) and old (age 26 mo) rats were rapidly excised of passive stiffness.

A custom-made computer program (LabVIEW, National Instruments) controlled muscle length during measurement of passive stiffness.

After training, the left and right Sol muscles from young adult (age 6 mo) and old (age 26 mo) rats were rapidly excised and placed in cooled (4°C) Krebs solution (2.5 mM Ca²⁺), aerated with 95% O₂-5% CO₂ (pH 7.35–7.4, P O₂ 400–460 Torr, P CO₂ 35–40 Torr). One muscle from each animal was then randomly chosen and vertically mounted in a glass tissue bath with circulating Krebs solution maintained at 26°C. The tendon from the origin of the muscle was attached to a stiff polycarbonate strip 1 mm in width, which in turn was secured to the lever arm of the Cambridge system. The tendon from the insertion of the muscle was clamped to a rigid metal rod, which in turn was fixed to a micropositioner, thereby allowing the length of the muscle to be incrementally adjusted to determine L₀ for peak twitch force (Pt). The muscle was stimulated via platinum electrodes (0.7 × 3 cm) using monophasic rectangular pulses (1.0-ms duration) of anodal current (Grass model S88 stimulator with current amplification). Stimulus intensity was increased until maximal Pt was obtained. L₀ for Pt was determined after supramaximal stimulation was achieved and was measured with a digital micrometer. Maximal isometric force was determined at varying frequencies ranging from 1 to 100 Hz, where tetanic stimuli were presented in 500-ms duration trains. Isometric force responses were displayed and measured on a digital storage oscilloscope (Hewlett-Packard).

Fig. 1. A: length changes of soleus muscle lengthened and shortened ±15% of optimal fiber length (Lₙ) at 1 Hz. A smaller (±0.5% Lₙ) oscillation at 75 Hz is superimposed on slower, larger oscillation. B: force changes of soleus muscle during change in muscle length.

Collagen hydroxyproline (HYP) assays. HYP assays were carried out on the Sol muscle (including tendons) from all four groups of rats. The HYP concentration was measured colorimetrically by using the method of Woenssner (31). Collagen concentration of each tissue sample was calculated with the assumption that collagen weighs 7.25× the measured weight of HYP (31).

HP cross-link assays. A portion of the prepared filtered acid hydrolyzate was used for HP cross-link analysis. The hydrolyzate was prepared for HP cross-link analysis by elution from a CF-1 cellulose (Sigma Chemical) column by using the procedure described by Skinner (25). The remaining hydrolyzate was concentrated, suspended in a slurry of CF-1 cellulose and n-butanol-acetic acid-water (4:1:1) mobile phase, and applied to the CF-1 cellulose column. HP, which absorbs onto organic acid-alcohol mixtures, was separated from the other amino acids and impurities eluting from the column with several column volumes of organic acid-alcohol mobile phase. HP was then eluted with water, and the fraction was dried in a Savant Speedvac for HP cross-link analysis. An aliquot of the dried hydrolysate from the CF-1 cellulose column was resuspended in 1% n-heptafluorobutyric acid (HFBA) containing pyridoxamine dihydrochloride (Sigma Chemical) as an internal standard. Pyridoxamine was used as an internal standard because of its structural and fluorescence similarities to HP. HP and pyridoxamine were detected by fluorescence spectrophotometry using excitation at 295-nm wavelength and emission at 395-nm wavelength. The isolation and quantification of the HP cross-link and pyridoxamine were accomplished by reverse-phase, high-performance liquid chromatography. The resolution of HP and pyridoxamine was obtained by using a binary mobile phase of 15% acetonitrile in water containing 0.01 M HFBA and 85% acetonitrile with 0.01 M HFBA. Identification of the HP peak was confirmed by comparison with a pure HP standard, which we routinely
EXERCISE AND MUSCLE STIFFNESS

Table 1. Effects of aging and exercise on whole body, soleus muscle, and left ventricular weights; on soleus-to-body weight and left ventricular-to-body weight ratios; and on maximal specific isometric force

<table>
<thead>
<tr>
<th>Group</th>
<th>BW, g</th>
<th>Sol, mg</th>
<th>LV, mg</th>
<th>Sol/BW, mg/g</th>
<th>LV/BW, mg/g</th>
<th>P&lt;oN/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>YC</td>
<td>392.7 ± 9.0</td>
<td>137 ± 3.2</td>
<td>609.1 ± 20.5</td>
<td>0.28 ± 0.01</td>
<td>1.58 ± 0.04</td>
<td>19.6 ± 0.5</td>
</tr>
<tr>
<td>YT</td>
<td>346.6 ± 6.1*</td>
<td>149 ± 2.3*</td>
<td>642.5 ± 10.6*</td>
<td>0.40 ± 0.02*</td>
<td>1.85 ± 0.02*</td>
<td>18.7 ± 0.4</td>
</tr>
<tr>
<td>OC</td>
<td>418.9 ± 26.2</td>
<td>125 ± 6.5†</td>
<td>744.7 ± 27.4†</td>
<td>0.23 ± 0.03</td>
<td>1.74 ± 0.10</td>
<td>14.8 ± 0.9†</td>
</tr>
<tr>
<td>OT</td>
<td>386.8 ± 7.7*‡</td>
<td>133 ± 4.4*‡</td>
<td>764.5 ± 23.9*‡</td>
<td>0.32 ± 0.03*</td>
<td>2.00 ± 0.07*</td>
<td>15.8 ± 0.7†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Shown are effects of aging and exercise on whole body weight (BW), soleus muscle weight (Sol), left ventricular weight (LV), ratio of Sol to BW (Sol/BW), ratio of LV to BW (LV/BW), and maximal specific isometric force (P<oN) of Sol muscles in young control (YC), young trained (YT), old control (OC), and old trained (OT) groups. *Significant main effect for exercise; trained significantly different from control groups (P < 0.05). †Significant main effect for age; old significantly different from young groups (P < 0.05).

RESULTS

Before training, same-aged groups were matched for body weight to evaluate the effects of the exercise program on whole body (BW), left ventricular (LV), and Sol muscle weights. A training effect was documented in both age groups of trained rats (YT, OT) compared with their sedentary counterparts (YC, OC) by attenuated weight gain and a relative hypertrophy of both LV and Sol muscles (Table 1). Maximal isometric-specific force was significantly lower (P < 0.05) in the Sol muscle of old compared with young rats and was unaffected by training (Table 1).

Stiffness of the Sol muscle for each group is illustrated in Fig. 2. At both 111 and 115% L₀, passive stiffness was significantly higher in the OC group compared with the YC and OT groups (Fig. 2, A and B; P < 0.05) but not with the YT group. A similar trend was observed at 107% L₀. Exercise training resulted in a significant decrease in the passive stiffness of the Sol muscle from old but not young adult animals.

Measurements of collagen concentration and cross-linking in the Sol muscle are shown in Figs. 3 and 4, respectively. Collagen concentration significantly increased in Sol muscle as a consequence of age. Exercise training had no effect on collagen concentration in the Sol muscle in either age group. There was a significant age-by-training interaction with respect to Sol muscle HP content. Intergroup comparisons revealed an age-associated increase in HP in OC compared with YC Sol muscle (0.77 ± 0.03 vs. 0.30 ± 0.03 mol HP/mol collagen; Fig 4). In the young rats, there was no training-associated change in HP content. In contrast, the OT Sol muscle HP content was significantly lower than that observed in the OC muscle (P < 0.0001).

DISCUSSION

The collagen data from the present study are consistent with previous reports. In both heart and skeletal muscle, aging results in an increase in collagen concentration and cross-linking (8, 28, 33). For example, when compared with young adult rats, collagen cross-linking is ~357, 27, and 380% higher in Sol muscle (33), diaphragm muscle (8), and LV free wall (28), respectively, of old rats. The increase in skeletal muscle collagen concentration seen with aging is not accompanied by a change in the activities of PH or galactosylhydroxylsine glucosyltransferase (11), two enzymes involved in posttranslational modification of collagen. Moreover, the fractional synthesis rate of collagen in rat skeletal muscle decreases ~10-fold, from 1 to 24 mo of age (17). These results suggest that the increase in collagen concentration observed in skeletal muscle from old animals is a result of a decreased rate of degradation out of proportion to the reduced biosynthetic activity.
The training protocol used in this study is similar to protocols used in other studies (7, 33) that resulted in significant increases in LV mass, maximal oxygen consumption, and locomotor muscle oxidative enzyme potential. In the present study, we observed a significant increase in collagen cross-linking in both senescent skeletal (33) and heart muscle (28). Because exercise training is associated with increased activity of PH and galactosylhydroxylysine glucosyltransferase in skeletal muscle (11) and heart (27), the reduction in collagen cross-linking in muscles of OT rats is presumably because of increased turnover rates of fibrillar collagen as a consequence of increased muscle recruitment.

We hypothesized that the reduction of collagen cross-links in trained muscle of old rats would significantly attenuate the increase in muscle stiffness. In the present study, muscle stiffness was significantly decreased in the OT compared with the OC group but was unchanged in the YT compared with the YC group. The reduced muscle stiffness in the OT group is consistent with the diminished content of HP cross-links seen in muscle from this same group. Our results differ from those reported by Kovanen and Suominen (10), who noted an increase in the passive stiffness of Sol muscles of OT rats. It is unclear why our results differ, but it may be due to a number of reasons. One possibility is that different training regimens were used (10 wk in our study vs. up to 2 yr in the study by Kovanen and Suominen). Because exercise intensity was not reported in the study by Kovanen and Suominen, it is not possible to comment on this aspect. We saw a significant reduction in the level of HP cross-links, whereas Kovanen and Suominen did not directly measure HP cross-links, but rather measured the amount of salt-soluble collagen. With the assumption that the level of HP cross-links corresponds to the amount of salt-soluble collagen, the training study by Kovanen and Suominen failed to result in a significant change in HP cross-links. Finally, all our mechanical measurements were initiated from \( L_0 \). Because Kovanen and Suominen initiated all measurements from an initial resting tension of 0.01 N rather than from \( L_0 \), it is unclear whether all muscles were at the same sarcomere length. A slight change in resting tension (or muscle length) can significantly alter the passive length-force curve.

The passive mechanical properties of skeletal muscle are similar to other soft tissues such that the passive load deformation curve is approximately exponential (9, 26). A number of variables may contribute to passive tension both at \( L_0 \) and at lengths above \( L_0 \). Studies employing isolated single fibers from frogs suggest that most of the resting tension is borne within the myofibril (15) and that the protein titin is involved in this phenomenon (30). However, in another study in which a muscle was passively stretched, the force-length curve was correlated with the amount of intramuscular connective tissue (9). In addition, differences in stress/strain properties have been noted between muscles with differing functional and collagenous properties (13). For example, slow-twitch muscle (Sol) has a greater collagen concentration and more extensive cross-linking of collagen than does a fast-twitch muscle (rectus femoris) (12). These biochemical properties of slow-twitch muscle correlate with a higher ultimate tensile strength (i.e., the point at which a muscle tears when stretched) than those of fast-twitch muscle. Additionally, fast-twitch muscle is capable of greater strain (i.e., change in length before mechanical failure) than is slow-twitch muscle (13). An even stronger argument for the importance of collagen cross-linking to tissue viscoelastic properties comes from studies in which the rate-limiting enzyme lysyl oxidase is inhibited by the presence of \( \beta \)-aminopropionitrile, thereby inducing lathyrism. Kovanen et al. (13) reported that

![Graph](http://jap.physiology.org/)

**Fig. 3.** Collagen concentration (means ± SE) of soleus muscle from YC, YT, OC, and OT groups. *Old significantly different from young, \( P < 0.05 \).

**Fig. 4.** Collagen hydroxylysylpyridinoline (HP) cross-link concentration (means ± SE) of soleus muscle from YC, YT, OC, and OT groups. *OC significantly different from YC, YT, and OT; \( P < 0.05 \).
both muscle tensile strength and stiffness significantly decrease under these conditions. Thus, although certain intracellular proteins no doubt contribute to passive viscoelastic properties of skeletal muscle, the extracellular matrix, and in particular collagen cross-linking, also has an important role in determining these parameters.

Despite the fact that OT animals were exercising at a much lower absolute workload than their younger counterparts, the training effect on SOL muscle HP cross-links was seen only in the older rats and not in the younger group, a finding observed previously (28, 33). These findings suggest that there is an optimal level of HP cross-link required for normal function of the muscle, and the level is maintained in skeletal muscle of young rats undergoing daily endurance exercise. Little is known, however, about the mechanisms that regulate collagen cross-linking in skeletal muscle. In cases of extreme copper deprivation or lathyrism, l-lysyl oxidase enzyme activity may be limiting. In muscle in which remodeling occurs, we have hypothesized that altered collagen fibril architecture, mediated by a small proteoglycan like decorin, may be a key collagen-binding regulator of cross-linking (20). It is also possible that mechanical load plays a role, because muscles with different functional properties have different levels of the HP cross-link (33).

It is presently unclear how much exercise (in terms of duration, frequency, and intensity) is required to achieve a reduction in muscle stiffness and HP cross-links in locomotor muscle from aged rats. The functional significance of reducing muscle stiffness in aging muscle by exercise training also has yet to be clarified. Because some studies have reported an increased susceptibility to exercise-induced injury in aging muscle (1, 32), one possible consequence of decreased stiffness may be reduced susceptibility to exercise-induced injury, particularly as a consequence of lengthening contractions. However, additional studies are required to test this hypothesis.

In conclusion, both muscle stiffness and the concentration of the mature collagen cross-link HP increases in SOL muscle of aging rats. However, regular exercise training can alter the passive mechanical properties of senescent SOL muscle, and this change is associated with concomitant changes in collagen cross-linking. These findings highlight the potential role of collagen in influencing the passive viscoelastic properties of skeletal muscle.

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REFERENCES


