Skeletal muscle collagen content in humans after high-force eccentric contractions

Abigail L. Mackey,¹ Alan E. Donnelly,¹ Taina Turpeenniemi-Hujanen,² and Helen P. Roper³

¹Department of Physical Education and Sport Sciences, University of Limerick, Limerick, Ireland; ²Department of Oncology and Radiotherapy, University Hospital of Oulu, Oulu, FIN-90020, Finland; and ³Department of Child Health, Birmingham Heartlands Hospital, Birmingham, B9-555, United Kingdom

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Mackey, Abigail L., Alan E. Donnelly, Taina Turpeenniemi-Hujanen, and Helen P. Roper. Skeletal muscle collagen content in humans after high-force eccentric contractions. J Appl Physiol 97: 197–203, 2004. First published February 27, 2004; 10.1152/japplphysiol.01174.2003.—The purpose of this study was to investigate the effects of high-force eccentric muscle contractions on collagen remodeling and on circulating levels of matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMP) in humans. Nine volunteers [5 men and 4 women, mean age 23 (SD 4) yr] each performed a bout of 100 maximum voluntary eccentric contractions of the knee extensors. Muscle biopsies were taken before exercise and on days 1, 2, 3, 4, 8, 11, and 14 postexercise. Venipuncture blood samples were also drawn on these days for measurement of serum creatine kinase activity and concentrations of MMP-9, TIMP-1, TIMP-2, and the MMP-2/TIMP-2 complex. Maximum voluntary contractile isometric force was recorded preexercise and on days 1, 2, 3, 4, 8, 11, and 14 postexercise. Venipuncture blood samples were also drawn on these days for measurement of serum creatine kinase activity and concentrations of MMP-9, TIMP-1, TIMP-2, and the MMP-2/TIMP-2 complex. Maximum voluntary contractile force declined by 39 ± 23% (mean ± SD) on day 2 postexercise and recovered thereafter. Serum creatine kinase activity peaked on day 4 postexercise (P < 0.01). Collagen type IV staining intensity increased significantly on day 22 postexercise to 126 ± 29% (mean ± SD) of preexercise values (P < 0.05). Serum MMP-9 levels increased on day 8 postexercise (P < 0.01), and serum TIMP-1 was also significantly elevated on days 1, 2, 3, 4, and 14 postexercise (P < 0.05). These results suggest that a single bout of eccentric muscle contractions results in remodeling of endomysial type IV collagen, possibly via the MMP pathway.

endomysium; type IV collagen; exercise; matrix metalloproteinases

UNACCUSTOED ECCENTRIC MUSCLE contractions in humans result in myofibrillar degeneration and subsequent regeneration, during which new myofibrils are formed (12). It is possible that such high-force muscle contractions also result in remodeling of the connective tissue component of muscle. Remodeling processes might act to repair possible direct damage to the endomysium resulting from high shear forces during eccentric contractions, or the remodeling could be secondary to the inflammation process occurring in the damaged muscle fibers. The muscle extracellular matrix is known to be dynamic in nature and is believed to respond to mechanical stress (19). Indirect studies of connective tissue breakdown products in human urine such as hydroxyproline and hydroxlysine suggest that these measures may change after high-force eccentric contractions (5) but not after downhill running (37). The response of endomysial collagen to exercise has not previously been examined directly in humans.

A close association has been demonstrated between increased collagen synthesis and the regenerative processes that occur in mouse skeletal muscle after exercise-induced muscle damage (26). Changes in immunohistochemical staining of muscle types III and IV collagen in that study were observed only in the endomysial connective tissue layer, with increased antibody staining evident from 5 to 20 days postexercise. Elevated levels of types I and III collagen mRNA have been observed in rat skeletal muscle in response to a bout of downhill running, a change that did not, however, result in detectable increases at the protein level (11). Basement membrane type IV collagen content has been shown to increase in rat skeletal muscle in response to lifelong endurance training (20). Furthermore, elevated mRNA levels of type IV collagen in rat skeletal muscle have been documented after an acute bout of exercise (11, 18); this was followed by augmented muscle type IV collagen content (18).

Matrix metalloproteinases (MMPs) are the primary agent through which degradation of extracellular matrix components is achieved. MMP-1 and -8 are generally considered to be the most effective at degrading the fibrillar types I and III collagen. The gelatinases, MMP-2 and -9, digest the denatured collagens and exhibit specificity toward certain native collagen types. MMP-9, for example, is capable of digesting type IV, XI, and XIV collagens, and MMP-2 is active against a range of collagens, including types III and IV (3, 36). Upregulation of both MMP-2 mRNA and enzymatic activity were detected in rat skeletal muscle in the days after a bout of running, and these values remained elevated at the last measurement 7 days after exercise (18). In the same study, both tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) and TIMP-2, inhibitors of MMP-2 and -9, were also upregulated during the degeneration and regeneration phases, respectively. Furthermore, it appears from myotoxin-induced muscle damage in murine skeletal muscle that expression of MMP-9 (produced by a variety of leukocytes) is associated with the early inflammatory response, whereas MMP-2 (produced mainly by fibroblasts) is involved in subsequent myofiber regeneration (16). TIMP-1 and -2 appear to follow a similar pattern (18). It has been reported that changes to circulating levels of MMPs and TIMPs are indicative of changes at the tissue level (15). An investigation into the serum MMP and TIMP antigenicity response to downhill running in humans revealed small but significant changes in...
levels of MMP-9, TIMP-1 and -2, and the MMP-2/TIMP-2 complex after the run; no change was observed in serum MMP-2 levels (17).

Previously published studies directly examining muscle collagen changes in response to exercise have been confined to the animal model (11, 18, 20). The aim of this study was to investigate the effects of a single bout of eccentric muscle contractions on muscle collagen in humans by using direct and indirect methods. We hypothesized that a bout of high-force eccentric muscle contractions would result in a transient increase in gelatinase activity, followed by accumulation of collagen types I, III, and IV in muscle endomysium. To our knowledge, the response of circulating levels of MMPs and TIMPs to high-force eccentric contractions has not been measured previously in humans. A second hypothesis of this study, therefore, was that serum antigenicity of MMP-9, TIMP-1, TIMP-2, and the MMP-2/TIMP-2 complex would be altered in response to the same exercise.

METHODS

Subjects

Nine healthy adults (5 men and 4 women) from the university population gave written, informed consent for the study, which had been approved by the University of Limerick Research Ethics Committee. Mean age, height, and weight of the volunteers was 23 \( \pm 4 \) (SD) yr, 1.7 \( \pm 0.1 \) m, and 71 \( \pm 12.6 \) kg, respectively.

Experimental Procedure

Each volunteer performed a series of 100 high-force eccentric muscle contractions of the knee extensor muscles of one randomly selected leg on an isokinetic dynamometer (CON-TREX MJ; CON-TREX AG, Dübendorf, Switzerland). The volunteers were required to maximally resist the forced lengthening of their quadriceps through a range of motion of 2.27 rad, from almost full extension to almost full flexion. Each contraction was performed at an angular velocity of 1.57 rad/s and was followed by a recovery phase during which the leg was returned to the starting position by the experimenter. The bout was divided into sets of six contractions with 30 s of rest between sets.

Microscopic Methods

Picrosiris red. Serial transverse sections (12 \( \mu m \)) were cut from the muscle samples in a cryostat at \(-18^\circ C\). The sections were stained with picrosiris red to visualize the presence of the peroxidase label. The original method was modified by the addition of fast blue, which has been reported to prevent uptake of Sirius red by the cytoplasm (38). The sections were placed in Bouin’s fluid (70% saturated aqueous picric acid, 5% acetic acid, 25% formalin) for 30 min. The slides were rinsed in distilled water for 1 min and placed in fast blue RR solution (0.15% fast blue RR, 7 mM magnesium sulfate, 60 mM magnesium borate) for 10 min. After three washes, the slides were placed in Picrosiris red solution (0.1% in saturated aqueous picric acid) for 20 min. A 10-s rinse in acidified water (0.5% glacial acetic acid in distilled water) was followed by a picric acid rinse (20% absolute ethanol, 70% distilled water, 10% saturated picric acid). The sections were dehydrated, cleared, and mounted.

Image analysis. Sections were viewed by light microscopy, and images were captured on a JVC TK-1280E digital color video camera (JVC), mounted on the microscope. Image acquisition was controlled through Image Grabber PCI software (Neotech, Eastleigh, Hampshire, UK). The 752 \( \times \) 582-pixel 24-bit RGB color images were saved as tagged-image format (TIFF) files, without compression. Contrast between the Sirius red and fast blue counterstain was enhanced in Adobe Photoshop (Adobe Systems, San Jose, CA), producing the same effect as introducing a green filter (wavelength of \( \sim 530 \) nm) into the light path of the microscope, as previously suggested (23). Each field covered an area of 0.64 \( \times \) 0.48 mm of section. Areas visibly containing perimysium were excluded from analysis. The mean area of tissue section analyzed per biopsy sample was 0.8 \( \pm \) 0.4 mm\(^2\) (mean \( \pm \) SD). Magnification for all images was \( \times 375\). The percent area of Sirius red staining within each section was calculated by applying an intensity threshold in SigmaScan Pro image analysis software (SPSS Science, Chicago, IL). The number of highlighted pixels was then expressed relative to the total number of pixels being evaluated.

Immunohistochemistry. Frozen sections were stained for types I, III, and IV collagen. The sections were fixed in precooled acetone for 10 min at \(-20^\circ C\). Goat serum (50 \% dilution) was added to the primary antibody (Rockland Immunocolors, Gilbertsville, PA), diluted in 1\% BSA-TBS, and left for 30 min at room temperature. The slides were washed thoroughly, and the sections were covered with diaminobenzidine substrate-chromogen (DAKO) for 12 min. The slides were washed before being

Serum Measurements

Blood sampling by venipuncture was carried out 3 days preexercise and on days 1, 2, 3, 4, 8, 11, and 14 postexercise, with blood being collected before the biopsy on days \(-3\) and \(+4\). Serum was collected after centrifugation and stored at \(-80^\circ C\) until analysis. Serum creatine kinase (CK) activity was measured with a standard enzymatic kit (Sigma no. 47-10; Sigma-Aldrich, Ireland, Dublin, Ireland). Serum concentrations of MMP-9, TIMP-1, TIMP-2, and the MMP-2/TIMP-2 complex were measured by standard sandwich ELISA protocols as described previously (40). Briefly, 96-well microtiter plates (Nunc Maxisorb, Nunc, Roskilde, Denmark) were coated with a monoclonal antibody against the analyte. Standards and samples were applied, and the bound analyte was detected with a horseradish peroxidase-conjugated secondary polyclonal antibody. Visualization of the presence of the peroxidase label was achieved by \(\alpha\)-phenylenediamine substrate. The color formation was measured by a microplate reader (Anthos 2000, Anthos Labtec Instruments, Salzburg, Austria) at 450 nm, and calculations were carried out using a Multicam program (Wallac, Turku, Finland).
Table 1. Serum CK activity, muscle soreness, and maximum voluntary contractile isometric force of the knee extensor muscles measured before and for 22 days after exercise

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<tr>
<td>CK activity, IU/l</td>
<td>216</td>
<td>562</td>
<td>997</td>
<td>6,495</td>
<td>18,129†</td>
<td>8,391</td>
<td>1,009</td>
<td>258</td>
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<td>Log of CK activity, log units</td>
<td>5.1</td>
<td>6.0*</td>
<td>6.4†</td>
<td>7.6†</td>
<td>8.4†</td>
<td>7.6†</td>
<td>6.2*</td>
<td>5.3</td>
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<td>Soreness, arbitrary units</td>
<td>0.8</td>
<td>0.8</td>
<td>1.1</td>
<td>1.6</td>
<td>2.0</td>
<td>2.1</td>
<td>1.3</td>
<td>0.8</td>
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<tr>
<td>MVC, N</td>
<td>621</td>
<td>392†</td>
<td>377†</td>
<td>377†</td>
<td>374†</td>
<td>408†</td>
<td>440†</td>
<td>452†</td>
<td>475†</td>
<td>527†</td>
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Values are means ± SD; n = 9 subjects. CK, creatine kinase; MVC, maximum voluntary contractile isometric force; Pre, before exercise; Post, after exercise. *P < 0.05, †P < 0.01 vs. preexercise, repeated-measures ANOVA. ‡P < 0.05 vs. preexercise, Wilcoxon’s signed-rank test.

dehydrated, cleared, and mounted. Control slides were processed in the same way as the experimental slides but with the omission of the primary antibody.

Image capture details were the same as those described above for Picrosirius red-stained sections. The mean total area of tissue section analyzed per biopsy sample was 1.2 ± 0.4 mm² (mean ± SD). The analysis of the images involved the use of a MATLAB (MathWorks, Natick, MA)-based algorithm (24), with modifications. Average staining intensity of three images from the negative control slide was subtracted from each result. Pearson’s correlation coefficient for the number of fibers and the staining intensity was calculated for each stain: type I collagen, 0.09; type III collagen, 0.06; type IV collagen, 0.17. The relationship between type IV collagen staining intensity and the number of capillaries was also investigated because of the presence of type IV collagen in capillary basement membrane. Pearson’s correlation coefficient for this relationship was −0.19. Neither this nor the correlations with fiber number was significant, indicating that the number of fibers and/or capillaries in a given field does not influence the individual collagen staining-intensity values.

In situ zymography. Visualization of tissue gelatinase activity was achieved by means of a recently published method (32) in which enzyme activity in the tissue cleaves a gelatin-fluorophore complex, releasing a fluorescent signal. Slides were immersed in preeqbal buffer (50 mM Tris, pH 7.4, 5 mM PMSF, 0.05% Brij 35) to remove embedding compound. Tissue sections were covered in commercially available substrate-fluorophore complex (gelatin from pig skin conjugated to Oregon green 488, Molecular Probes Europe, Leiden, The Netherlands), diluted 1:10 in substrate buffer (50 mM Tris, pH 7.4, 5 mM PMSF, 0.05% Brij 35, 10 mM calcium chloride), and incubated for 4 hours in darkness. The slides were washed three times in distilled water and immersed in 0.5% Triton X-100 for 10 min, followed by three distilled water washes. ProLong Antifade (Molecular Probes Europe) was the mounting medium employed. Negative control slides were included for each biopsy sample and treated in the same way as the experimental slides but with the omission of gelatin. Images were captured on a Photometrics CoolSNAP digital camera (Roper Scientific, Trenton, NJ) mounted on the microscope. Image acquisition was controlled through Metamorph Imaging System software (Universal Imaging, Downingtown, PA). All images were captured with the same exposure setting of 2 s. The 1,300 × 1,030-pixel 16-bit gray-scale images covered an area of 0.45 × 0.36 mm² of tissue section and were saved in TIFF format. The mean area of tissue section analyzed per biopsy sample was 0.9 ± 0.3 mm² (mean ± SD). The final magnification of the images was ×900. Endomyosial staining intensity was measured as described above for the immunohistochemical method.

Statistical Analysis

Parametric data were analyzed using repeated-measures ANOVA (SuperANOVA, Abacus Concepts, Berkeley, CA) with changes over time as the within-subject factor. Where a significant difference over time was indicated, a means-comparison test was carried out to determine which time points were different from the preexercise value. Effect size was also calculated by using the pooled standard deviation, and the G*Power statistical software program (8) was used to calculate statistical power for the ANOVA tests. The nonparametric soreness data were analyzed by Wilcoxon’s signed-rank test. Differences were considered statistically significant at $P < 0.05$. Data are presented as means ± SD, unless otherwise stated.

RESULTS

Indirect Assessment of Muscle Damage

Indirect indexes of muscle damage are presented in Table 1. MVC force decreased immediately postexercise and remained below the preexercise values of 621 ± 153 N (mean ± SD) for days 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 14, and 22 postexercise ($P < 0.01$). Force decline was maximal on days 2 and 3 postexercise (39 ± 23 and 39 ± 25% of the preexercise values, respectively). Muscle soreness scores increased, with the highest scores of 20 ± 8 and 20 ± 9 also occurring on days 2 and 3 postexercise ($P < 0.05$). Serum CK activity levels were elevated from 216 ± 149 to 18,129 ± 31,382 IU/l on day 4 postexercise ($P < 0.01$). There was large variability in the serum CK response to the eccentric contractions, although all volunteers demonstrated an increase (minimum value on day 4: 357 IU/l, maximum value 98,725 IU/l). A logarithmic transformation was carried out on the CK data as a result of the large variability. Statistical analysis of this data revealed significant increases in serum CK activity on days 2, 3, 4, and 8 postexercise compared with preexercise levels ($P < 0.01$). The activity levels on days 1 and 4 postexercise were also significantly elevated ($P < 0.05$).

Tissue Analysis

Tissue gelatinase activity and the percent section area stained with Sirius red did not change significantly for all biopsy time points postexercise (Table 2). Immunohistochemical staining results are displayed in Fig. 1. The intensity of type IV collagen changed significantly over time ($P < 0.05$, ANOVA), increasing to a value of 126 ± 31% of preexercise levels on day 22 postexercise. Post hoc means-comparison tests indicated that the 22-day sample was significantly greater than both the preexercise sample ($P = 0.01$) and the day 4 postexercise sample ($P = 0.04$), but the day 4 postexercise sample did not differ significantly from preexercise. There was also a significant correlation between serum CK activity and
TIMP-1 levels remained above the preexercise values for days 4 to 103 ng/ml within 24 h postexercise (\(P<0.05\), repeated-measures ANOVA. Serum MMP and TIMP data are displayed graphically in Fig. 2. Circulating levels of MMP-9 increased from 112 ± 42 ng/ml preexercise to 188 ± 49 ng/ml on day 8 postexercise (\(P<0.01\)). Serum TIMP-1 increased from 344 ± 91 to 439 ± 103 ng/ml within 24 h postexercise (\(P<0.001\)). Furthermore, TIMP-1 levels remained above the preexercise values for days 2, 3, 4, and 14 postexercise (\(P<0.05\)). There was no change in circulating levels of either TIMP-2 or the MMP-2/TIMP-2 complex.

### DISCUSSION

The results of the present study show significantly increased staining intensity of type IV collagen in human muscle endomysium 22 days after a single bout of unaccustomed high-force eccentric muscle contractions. Measured values for type III collagen immunohistochemical staining and total collagen recorded on day 22, although elevated, were not significantly different from baseline at the \(P<0.05\) level on the basis of this statistical test. Because the sample size was low, with subsequent potential for type II error, values for effect size and statistical power have also been calculated for the immunohistochemical and histochemical measurements. Moderate to large effect sizes for type III collagen immunohistochemical staining and total collagen staining were observed when baseline was compared with day 22 (Table 3), suggestive of a trend toward increase for these values on day 22, but the statistical power resultant from an \(n\) value of 9 meant that these trends did not achieve statistical significance in this study. Circulating levels of MMP-9 and its inhibitor TIMP-1 were significantly altered postexercise. These findings together suggest that myofiber basement membrane undergoes remodeling via the MMP pathway in response to muscle damaging exercise.

The responses of the indirect indexes of muscle damage measured in this study (MVC force, muscle soreness, and muscle collagen IV content on day 4 postexercise (\(r = 0.854, P = 0.003\)). Types I and III collagen staining intensity, relative to preexercise levels, did not change significantly. The mean staining intensity values for type III collagen were 130 ± 70.6 and 150 ± 96.9% of preexercise values on days 4 and 22 postexercise, respectively. When expressed relative to preexercise staining levels, values ranged from 93 to 307 and from 87 to 382% on days 4 and 22 postexercise, respectively. Given this large variation between individuals and low subject number, effect size was also calculated (Table 3). Moderate effect sizes were observed between the preexercise sample and both the day 4 and day 22 samples for percentage area of Sirius red staining and percentage changes in type III collagen staining intensity and gelatinase staining intensity. A large effect size was observed for the percentage increase in type IV collagen staining intensity 22 days postexercise compared with the preexercise mean.

### Table 3. Effect size and statistical power for histochemical staining data from muscle biopsy time points, calculated using the pooled standard deviation

<table>
<thead>
<tr>
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<th>Pre to Day 4</th>
<th>Day 4 to Day 22</th>
<th>Pre to Day 22</th>
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<tbody>
<tr>
<td>Collagen I</td>
<td>-0.23 (0.21)</td>
<td>0.41 (0.28)</td>
<td>0.35 (0.30)</td>
</tr>
<tr>
<td>Collagen III</td>
<td>0.57 (0.46)</td>
<td>0.23 (0.21)</td>
<td>0.73 (0.57)</td>
</tr>
<tr>
<td>Collagen IV</td>
<td>0.24 (0.22)</td>
<td>0.66 (0.52)</td>
<td>1.17 (0.78)</td>
</tr>
<tr>
<td>Sirius Red</td>
<td>0.49 (0.57)</td>
<td>0.40 (0.33)</td>
<td>0.84 (0.55)</td>
</tr>
<tr>
<td>Gelatinase</td>
<td>0.45 (0.38)</td>
<td>0.06 (0.09)</td>
<td>0.67 (0.53)</td>
</tr>
</tbody>
</table>

Sirius red data used were the percent area of tissue stained with Sirius red; collagen types I, III, and IV and gelatinase data were intensity of staining, expressed relative to preexercise values. An effect size (ES) of 0.8 or greater is large, an ES of ~ 0.5 is moderate, and an ES of 0.2 or less is small (35). Statistical power (1 − \(\beta\)) for each effect size is shown in parentheses.
serum CK activity) were characteristic of those after unaccustomed high-force eccentric muscle contractions (4, 5, 27, 28), with high serum CK variability between individuals. These results would suggest that the damage to the relatively small muscle mass employed in this study was more intense than that after other damaging models such as downhill running, where lower CK values are generally obtained, and where a larger muscle mass is employed during exercise, suggesting more diffuse damage. It was noteworthy that muscle force had not fully recovered 22 days after the bout, indicating that the effects on muscle force were both severe and more prolonged than is generally observed in similar studies.

**Collagen IV**

The increase in type IV collagen staining intensity on day 22 after the exercise was not related to capillary number, nor with the number or type of fibers in each image, suggesting that this result is not artifactual. Type IV collagen has been reported to be upregulated at the gene level in rat muscle as early as 6 h after downhill running, mRNA levels remaining elevated for 7 days postexercise (18). In that study, differences in type IV collagen content were associated with fiber type and/or the extent of muscle fiber damage sustained. In the present study, the strong correlation between a marker of muscle fiber damage, serum CK activity, and type IV collagen staining on day 4 would suggest that the eccentric muscle contractions had disturbed collagen IV turnover.

**Endomysial Fibrillar Collagens**

In a study of downhill running in rats, Han et al. (11) reported upregulation of types I and III collagen at the gene level with increased synthetic enzyme activity, although there was no change in the protein level of either collagen type. In the present study, there were no statistically significant changes in intensity of endomysial staining for either type I or type III collagen. Although there was large individual variation in type III collagen staining intensity after the bout, the mean increase did not reach significance with ANOVA, and it is notable that the collagen III content actually decreased in some individuals. However, effect size calculations for type III collagen staining intensity indicate a moderate effect by day 4 and a larger effect by day 22 postexercise.

**Total Collagen**

The relative area of total endomysial collagen, measured by image analysis of Sirius red-stained tissue, did not change significantly for the time points measured in this study, although a large effect size was observed between the preexercise and day 22 samples. The Sirius red method has been widely used for collagen staining in tissue sections (14, 34), although some doubt exists as to its specificity for collagen alone; binding to other proteins containing basic amino acids has been demonstrated (29). Previously, it has been successfully used to detect changes in the cross-sectional area of collagen in aging and dystrophic mice (10) and after periods of limb distraction (38) and immobilization (21, 39).

**Serum MMPs**

Increased serum MMP-9 and TIMP-1 concentrations post-exercise were detected by ELISA in the present study. Although no changes were observed in circulating levels of TIMP-2 or the MMP-2/TIMP-2 complex, greater serum MMP-9 levels were observed on day 8 postexercise, whereas serum TIMP-1 levels rose on day 1 postexercise and remained elevated on days 2, 3, and 14 postexercise (Fig. 2). TIMP-1 binds MMP-9, blocking enzyme activity, and pro-MMP-9, preventing activation of the enzyme (9). The ELISA method employed in the present study detects both of these complexes as well as the free form, so it was not possible to distinguish between these forms in this study. The prolonged increase in serum TIMP-1 levels observed up to day 14 postexercise is suggestive of sustained regulation of MMP activity. Because there was no change in circulating levels of TIMP-2, the more potent MMP-2 inhibitor, or the MMP-2/TIMP-2 complex, it is likely that changes to TIMP-1 presented here are related to MMP-9 activity. The difference in the time course of the changes observed in circulating TIMP-1 and MMP-9 may reflect different rates of uptake and/or release by the tissues.

The immediate and transient increase in serum MMP-9 concentration observed by Koskinen et al. (17) was suggested to be the result of increased mobilization of leukocytes, a phenomenon observed in response to downhill running in humans (30). Extensive MMP-9 upregulation has been observed after myotoxin-induced damage (16) but not exercise-induced damage (18). In the myotoxin-induced damage study, MMP-9 was localized to polymorphonuclear leukocytes and macrophages, its expression being attributed to the acute inflammatory response (16). In contrast to this, serum MMP-9 levels in the present study did not show any sign of increase until the 8th day postexercise.

**TIMPs**

The intensity of endomysial staining of gelatinase activity (combined MMP-2 and -9 activity) was measured by quantitative image analysis. No overall significant change in enzyme activity was detected on day 4 or 22 postexercise, although some individuals did show increases of up to 50% in gelatinase activity on day 22, and this is supported by moderate effect size values. The turnover of basement membrane type IV collagen observed by Koskinen et al. (18) in rat skeletal muscle was accompanied by increased activity of MMP-2 and its inhibitor, TIMP-2. MMP-9, the other potent type IV collagen-degrading enzyme, was below the detection threshold in that study, at both the mRNA and protein levels.

**Stimulus for Remodeling**

Limb immobilization has been reported to result in often dramatic increases in muscle collagen content (39). Lapier et al. (21) also reported accumulation of endomysial collagen after a period of limb immobilization in the lengthened position and observed that the immobilization of the muscle appeared protective against subsequent contraction-induced damage. However, a study of limb suspension immobilization in humans found that it made the muscle more susceptible to damage (31), although the limb was not immobilized in the lengthened position. It has been noted that limbs immobilized with the muscle in the lengthened position result in greater increases in muscle collagen content (39), indicating that stretch could be a stimulus for collagen proliferation. Results from other studies are, however, conflicting. Muscles immobi-
lized in the lengthened position (13) and the shortened position (13, 39) have both resulted in increased muscle collagen content, whereas no change was detected in muscle immobilized in the middle position (33). It has been also been reported that immobilization of rat skeletal muscle results in downregulated synthesis of types I, III (2), and IV collagen (1). The study reported here does not allow the mechanism for collagen IV change to be elucidated, and it is not clear whether the stimulus for collagen remodeling was physical disruption of the endomysium, high mechanical strain on the muscle, or subsequent damage-related inflammatory events. The eccentric protocol employed in this study was similar to that employed in a prior human study in which changes in excretion of hydroxyproline and hydroxylysine were observed (5), and it is possible that the endomysial response observed in the present study and the changes in collagen breakdown described in the prior study occurred in response to a severe eccentric insult to the muscle, which might not be duplicated after less damaging exercise.

Potential Limitations of the Study and Potential for Further Investigation

Muscle tissue sampling using the needle biopsy technique is a common procedure, but it is possible that the connective tissue content varies from biopsy to biopsy. It has been reported that, in human vastus lateralis muscle, most of the variability in muscle fiber area is a function of depth (22). The location on the thigh and the depth to which the biopsy needle was inserted were kept constant for all subjects in the present study. Furthermore, it has also been reported that a typical human muscle needle biopsy is sufficient to characterize fiber area and capillarity of an individual (25). Clearly, the biopsy technique limits the opportunity to collect samples and allowed only a limited time course to be observed. Another feature of these results is the quantification of staining intensity in tissue sections. Digital image analysis is semiquantitative, true quantification of a protein requiring analysis of tissue homogenate. The localization of the proteins is, however, lost in this process, and it becomes no longer possible to identify changes specific to endomysial or perimysial layers individually. Because the endomysium is a relatively fine structure, the perimysium could easily mask changes at the endomysial level when the tissue is analyzed in its homogenate form.

As far as we can ascertain, this is the first description of changes in human intramuscular collagen IV after exercise. It would be interesting to observe the time course and duration of the change and to elucidate whether it is a feature of muscle-damaging exercise or whether it occurs after nondamaging training. Furthermore, the effects of muscle contraction on endomysial total collagen and on endomysial collagen III content need further investigation.

In conclusion, the results presented in this paper provide original evidence of increased type IV collagen staining intensity in human muscle endomysium after a bout of high-force eccentric muscle contractions, suggestive of extracellular matrix remodeling. This result is augmented by the altered levels of circulating MMP-9 and TIMP-1 postexercise. Together, these results imply that a single bout of muscle contractions can elicit a prolonged change in the extracellular matrix composition within skeletal muscle. Further work is required to elucidate the duration of this effect and to discover whether the stimulus for the change was mechanical or metabolic in origin.

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