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The Effect of Corticosteroid on Collagen Expression in Injured Rotator Cuff Tendon

Anthony S. Wei, MD,

Department of Orthopaedic Surgery and Rehabilitation, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153

John J. Callaci, PhD,

Department of Orthopaedic Surgery and Rehabilitation, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153

Dainius Juknelis, MD,

Department of Orthopaedic Surgery and Rehabilitation, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153

Guido Marra, MD,

Department of Orthopaedic Surgery and Rehabilitation, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153

Pietro Tonino, MD,

Department of Orthopaedic Surgery and Rehabilitation, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153

Kevin B. Freedman, MD, MSCE, and

Orthopaedic Specialists, 27 South Bryn Mawr Avenue, Bryn Mawr, PA 19010

Frederick H. Wezeman, PhD

Department of Orthopaedic Surgery and Rehabilitation, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153

Anthony S. Wei: awei@lumc.edu

Abstract

Background—Subacromial corticosteroid injections are commonly used in the nonoperative management of rotator cuff disease. The effects of corticosteroid injection on injured rotator cuff tendons have not been studied. Our aims were to characterize the acute response of rotator cuff tendons to injury through the analysis of the type-III to type-I collagen expression ratio, a tendon injury marker, and to examine the effects of corticosteroid on this response.

Methods—Sixty Sprague-Dawley rats were randomly assigned to four groups: control, tendon injury, steroid treatment, and tendon injury and steroid treatment. Six rats served as sham controls. Unilateral tendon injuries were created with full-thickness defects across 50% of the total width of the infraspinatus tendon, 5 mm from its humeral insertion. Steroid treatment with a single dose of methylprednisolone (0.6 mg/kg), equivalent to that given to humans, was injected into the subacromial space under direct visualization. Steroid treatment followed the creation of an injury in the rats in the injury and steroid treatment group. At one, three, and five weeks after the injury, the total RNA isolated from tendons was quantified with real-time polymerase chain reaction with use of primers for type-I and type-III collagen and ribosomal 18s RNA.

Results—The type-III to type-I collagen expression ratio remained at baseline at all time-points in the control and sham groups. At one week, the type-III to type-I collagen expression ratio increased more than fourfold above the control level in the tendon injury group ($p = 0.017$) and the

tendon injury and steroid treatment group ($p = 0.003$). The ratio remained greater than twofold above the control at three weeks in both groups ($p = 0.003$ and $p = 0.037$) and returned to baseline at five weeks. Interestingly, the group that had steroid treatment only showed an increase of >4.5-fold ($p = 0.001$) in the type-III to type-I collagen expression ratio, without structural injury to the tendon. This ratio returned to baseline levels by three weeks.

Conclusions—A single dose of corticosteroid does not alter the acute phase response of an injured rotator cuff tendon in the rat. However, the same steroid dose in uninjured tendons initiates a short-term response equivalent to that of structural injury.

Clinical Relevance—These findings suggest that while a single corticosteroid dose may have no long-term effects on tendon collagen gene expression, collagen composition may be acutely altered by the injection. Therapy and activity recommendations following subacromial corticosteroid exposure should be made with the awareness of possible compromised rotator cuff tendon properties.

Conservative measures are the mainstay in the treatment of a spectrum of rotator cuff disease ranging from tendinosis to partial-thickness tears^{1,2}. Nonoperative treatment options, including rest, activity modification, nonsteroidal anti-inflammatory medications, physical therapy, and subacromial corticosteroid injections, are all used in the initial management of rotator cuff abnormalities prior to early surgical intervention³. Studies have demonstrated clinical improvements in pain relief and range of motion when subacromial corticosteroid injections are used in the treatment of rotator cuff tendinosis^{4–6}.

The beneficial anti-inflammatory and analgesic properties of corticosteroid are tempered by potentially important side effects on connective tissue. There are numerous clinical reports of spontaneous tendon ruptures following the use of local corticosteroid injections or systemic steroids^{7–9}. Animal studies performed primarily on the Achilles tendon, the patellar tendon, and the medial collateral ligament of the knee have found corticosteroid exposure to be associated with tendon and ligament atrophy, fragmentation of collagen bundles, decreased biomechanical properties, and delayed healing^{10–13}. Other experimental studies, however, have yielded no clearly deleterious effect following local corticosteroid exposure^{14–17}. The lack of consensus in the literature, combined with possible important sequelae, has led to a cautious use of subacromial corticosteroid injections in the clinical setting^{3,5,18}.

The specific impact of corticosteroid on the rotator cuff tendon remains poorly understood. It is difficult to apply conclusions from other tendon studies to the rotator cuff because of its unique location between the coracoacromial arch and subacromial bursa superiorly and the glenohumeral articulation inferiorly. This distinctive anatomy plays an important role in rotator cuff tendon disease and must be considered when analyzing local corticosteroid effects¹⁹. Previous anatomic studies of the rat shoulder have demonstrated a close structural relationship to the human shoulder^{20,21}. The rat rotator cuff model has since been established as an *in vivo* model for studying rotator cuff disease^{22–24}. Several investigators have used this model to evaluate the specific *in vivo* effects of sub-acromial corticosteroid injection on rotator cuff tendons^{25,26}. Those studies demonstrated histologic evidence of tendon injury to healthy, intact tendons following multiple subacromial corticosteroid injections given at short intervals. While important, the studies did not reflect the clinical use of corticosteroids for diseased rotator cuff tendons. To our knowledge, no study to date has examined the effects of subacromial corticosteroid injections on injured rotator cuff tendons.

Type-I collagen, the most abundant collagen found in normal tendons, makes up >90% of the total collagen content of a tendon^{27,28}. When tendons are injured, an increased

proportion of type-III collagen is produced during the early injury response. As injured tendons remodel and mature, the baseline type-III to type-I collagen ratios are gradually restored²⁹⁻³¹. The acuity of this response makes the type-III to type-I collagen ratio a sensitive marker for tendon injury^{31,32}.

The purpose of this study was to create an acute injury in the rat infraspinatus tendon and to characterize the molecular response to the injury and corticosteroid treatment with use of the ratio of type-III to type-I collagen gene expression. The hypothesis of this investigation was that corticosteroid treatment alters the collagen gene expression in the rotator cuff during an acute injury response.

Materials and Methods

Rat Surgical Model

Sixty Sprague-Dawley male rats, with a mean body weight of 570 g (range, 422 to 700 g), were used in this study, which was approved by the Institutional Animal Care and Use Committee. The rats were randomly assigned to one of four groups: control (Group 1), tendon injury (Group 2), steroid treatment only (Group 3), and tendon injury and steroid treatment (Group 4). Each group consisted of eighteen shoulders. Six rats served as sham surgery controls. Groups 1 and 2 were composed of the same rats, with the right shoulders and left shoulders, respectively, used in each group. The left shoulders were used in both of the remaining groups.

We selected the infraspinatus tendon to study rotator cuff tendon injury and the effects of subacromial corticosteroid exposure. Schneeberger et al. found that the rat infraspinatus tendon was not only substantially longer than the supraspinatus tendon but also in close contact with the overlying acromial arch across its entire width²³. The fact that the rat infraspinatus tendon has a greater amount of working tendon tissue than the supraspinatus and that it has an anatomic relationship comparable with that of the human rotator cuff and coracoacromial arch made it an ideal model for our study.

Surgical Procedure

A subcutaneous injection of antibiotics (8 mg/kg of gentamicin) was given preoperatively. Anesthesia was induced through intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). The sterile operations were performed under loupe magnification. A 1-cm transverse skin incision was made along the lateral border of the acromion. A portion of the deltoid origin was sharply detached from the acromion. The acromion was then gently retracted, exposing the infraspinatus tendon. Unilateral tendon injuries were created with full-thickness defects across 50% of the total width of the infraspinatus tendon, 5 mm from the humeral insertion (Fig. 1). In the rats in Group 2 (tendon injury), the wound was then closed without corticosteroid exposure. For closure, the fascia of the detached deltoid muscle was sutured to that of the trapezius muscle, and the skin was closed with surgical staples. No activity restrictions were imposed following surgery.

In the rats exposed to steroid (Groups 3 and 4), a single dose of methylprednisolone acetate (0.6 mg/kg) that was equivalent to that given to humans was injected, with use of a sterile micropipette, into the open subacromial space under direct visualization. In Group 4, the steroid treatment directly followed the creation of the tendon injury and occurred prior to closure of the subacromial space. In Group 3, the corticosteroid injection was placed in the open subacromial space following exposure of the infraspinatus tendon, without a tendon defect being created. In both groups, local extravasation of corticosteroid was not noted following the injection. The rat shoulders were then closed as described above.

The sham surgery group underwent an identical exposure of the left infraspinatus tendon followed by a subacromial injection of saline solution. The volume of saline solution used was determined by calculating the volume of methyl-prednisolone acetate that each rat would have received in the corticosteroid injection groups.

At one, three, and five weeks after surgery, the rats were killed with use of a CO₂ chamber. The infraspinatus tendon was harvested from six rats in each group at each time-point. Care was taken to separate the tendon from any muscle or overlying bursal tissue. Efforts to maximize the tissue yield for RNA analysis were made by elevating the tendon directly off its humeral insertion and amputating it at the musculotendinous junction proximally. The infraspinatus tendons were immediately snap-frozen in liquid nitrogen and stored at -80°C.

Collagen Gene Expression Analysis

Isolated tendon tissue was first disrupted with use of a Spex Freezer/Mill (Spex CertiPrep, Metuchen, New Jersey). Total RNA was extracted from pulverized tendons with use of the RiboPure isolation system (Ambion, Austin, Texas) according to the manufacturer's instructions. Real-time polymerase chain reaction was performed on total RNA samples with use of the Assays-on-Demand Gene Expression System (Applied Biosystems, Foster City, California). Briefly, 2.5 µg of total RNA was used for reverse transcription to generate cDNA. The thermal cycler program was one cycle at 25°C for ten minutes and then 37°C for 120 minutes. Real-time polymerase chain reaction analysis was then performed with use of 50 ng of cDNA amplified with primer-probe sets specific for type-I (α_2 [I] chain) collagen, type-III (α_1 [III] chain) collagen, and ribosomal 18s RNA. The cyclic program consisted of 95°C for ten minutes followed by forty cycles at 95°C for fifteen seconds and 60°C for one minute. Collagen mRNA levels were individually normalized to ribosomal 18s levels.

Statistical Analysis

Statistical analysis was performed with Minitab 13.2 software (Minitab, State College, Pennsylvania). A power analysis was performed to estimate the number of biological replicates that would provide reasonable power (>80%) to distinguish control and treatment groups. Collagen gene expression values were normalized to the control group for each experimental time-point. The type-III to type-I collagen mRNA ratio was calculated for each rat tendon. Data were analyzed by one-way analysis of variance followed by the Dunnett and Tukey multiple comparison procedures. The level of significance was set at $p < 0.05$.

Results

The rats in the present study showed no adverse effects related to the surgery or subacromial corticosteroid exposure. None of the animals had signs of local or systemic infection develop. Postoperatively, the rats exhibited normal gait patterns and had full use of the involved forelimb within three to four days. Following the initial recovery period, the animals all displayed preoperative levels of activity and food intake.

Macroscopic Tendon Morphology

Macroscopically, all tendons from the control group demonstrated normal structure. The rats in Group 3 (steroid treatment only) had softer and duller tendons; this appearance was the most obvious at one week after the injection. Tendons harvested from the rats in both Group 2 (tendon injury) and Group 4 (tendon injury and steroid treatment) had evidence of neovascularization that extended from proximal to distal along the bursal surface. This finding was most prevalent during the first and third week after the injury. Additionally, the tendon defects in both groups were filled with granulation tissue that spanned the defect by

one week after the injury. No appreciable morphologic difference, however, could be appreciated between the two groups at the macroscopic level.

Type-I Collagen Expression

The type-I collagen mRNA expression levels for all groups, at each time-point, were reported as a percentage of that of the control group, which was set at 100% (Table I). The expression of type-I collagen did not increase above the control levels until five weeks after the injury in both Groups 2 ($p = 0.05$) and 4 ($p < 0.05$). A similar trend was also present in Group 3 (steroid treatment only) ($p < 0.05$), despite the lack of any physical injury to the infraspinatus tendon in these rats. In addition, the magnitude of the increase in expression was the same across all three groups at five weeks.

At the three-week time-point, the mRNA expression level of type-I collagen in Groups 2, 3, and 4 was not appreciably different from the Group-1 (control) baseline. However, type-I collagen expression levels were almost twofold higher in Group 3 ($p = 0.043$) and Group 4 ($p = 0.025$), the steroid-treated groups, compared with Group 2 (tendon injury).

Type-III Collagen Expression

The type-III collagen mRNA expression in Groups 2, 3, and 4 was similarly reported as a percentage of that of the control group (Table II). Unlike the type-I collagen expression pattern, type-III collagen mRNA in all three groups showed a dramatic fourfold to fivefold increase ($p < 0.05$) in the first week following injury and/or steroid exposure. Expression then remained significantly above control levels for all groups through the third and fifth week.

In Group 2 (tendon injury), the mRNA expression levels demonstrated a temporary decrease at three weeks that was no longer apparent by five weeks. This reduced expression was also seen in Group 4 (tendon injury and steroid treatment) at the third week. However, the magnitude of the decrease was significantly greater in Group 2 ($p < 0.05$).

Type-III to Type-I Collagen Expression Ratio

The type-III to type-I collagen expression ratio was calculated on the basis of the individual type-I and type-III collagen mRNA expression data (Fig. 2). In the sham surgery group, the type-III to type-I collagen expression ratio remained unchanged from the control group ratio at all three time-points. At one week, the type-III to type-I collagen expression ratio for Groups 2 and 4 increased more than fourfold ($p = 0.017$ and $p = 0.003$, respectively) compared with the control group. The ratio for both groups remained greater than twofold ($p = 0.003$ and $p = 0.037$, respectively) that of the control group at three weeks and returned to the control level at five weeks. Group 3 demonstrated a similar fivefold increase ($p = 0.001$) in the type-III to type-I collagen expression ratio at one week. But unlike Groups 2 and 4, the elevated expression ratio returned to control levels by the third week.

Discussion

Subacromial corticosteroid injections are commonly used in the nonoperative management of rotator cuff diseases^{5,18,33}. Current clinical recommendations often limit their use to no more than two or three injections a year, spaced three or more months apart^{3,34}. The caution surrounding corticosteroids stems largely from the potential side effects that have been reported in the literature. Tendon rupture, connective tissue atrophy, and decreased tendon biomechanical properties have all been associated with local corticosteroid exposure^{7-13,35}. However, the literature is largely made up of anecdotal clinical case reports and animal studies that have usually used Achilles tendon or patellar tendon models. Furthermore,

numerous clinical and animal studies have also shown that peritendinous corticosteroid exposure causes no definitive adverse effects on tendon^{15–17,36,37}. The absence of a clear consensus, as well as a lack of studies specifically on the effect on the rotator cuff tendon, underlies the current empiric nature of guidelines for subacromial corticosteroid use.

Few studies to date have examined the specific clinical effects of corticosteroids on rotator cuff tendons. The clinical studies that are available are often limited to retrospective case series. Watson noted that four or more preoperative corticosteroid injections were associated with a “softer” residual cuff tissue, as determined intraoperatively, and inferior outcomes following open rotator cuff repair³⁸. That study, however, did not describe the details of the injections, including the dose, type of corticosteroid used, or the interval at which the corticosteroid was injected. Bjorkenheim et al. also found a higher rate of repair failures in patients who had received more than three preoperative injections of corticosteroid³⁹, but, again, the nature and details of the injections were not described.

Individual Type-I Collagen and Type-III Collagen mRNA Expression

In our study, a distinct temporal change in collagen expression was observed following the creation of the tendon injury. At one week after the injury, infraspinatus tendons from Group 2 (tendon injury) exhibited a significant increase in type-III collagen mRNA expression. The levels then remained above the control group expression level through the fifth week. The same tendons, however, did not exhibit a significant increase in type-I collagen mRNA expression until five weeks after the injury. This variation in collagen type expression is consistent with previous models of tendon injury, in which type-III collagen is produced preferentially over type-I collagen during the early phase of injury response^{29–31,40}. Cheung et al. found that type-III collagen contains a high number of interfibrillar cross-links that can be generated rapidly, providing provisional strength to injured tendons⁴¹. As the tendons mature, type-I collagen gradually replaces the smaller (<60 nm) reticular fibrils of type-III collagen^{29,31,42}. Type-I collagen, which normally makes up >95% of normal tendon collagen, then provides the majority of the tensile strength of the tendon through thick (>100 nm), densely packed, parallel fibrils^{29,42,43}.

The presence of subacromial corticosteroid did not change the overall tendon response in Group 4 (tendon injury and steroid treatment). The type-I collagen expression level remained at baseline until increasing sharply at five weeks after the injury, while the type-III collagen expression elevated significantly and remained above the control level from the first week on. But even though the general temporal trend of type-I collagen and type-III collagen expression was unaffected by subacromial corticosteroid exposure, a noticeable variation in the expression of both collagen types was apparent among the groups at week three. A twofold increase in the expression of type-I collagen was seen in Group 4 compared with Group 2. Although this increased level was not significant over the control expression at one week, it was also evident in Group 3 (steroid treatment) at three weeks. This would suggest that the elevated expression of type-I collagen is likely a corticosteroid-induced effect that occurs independent of tendon injury. The clinical importance of this finding, however, remains unknown, given that the expression level of type-I collagen was ultimately equivalent across all three groups at five weeks.

Type-III collagen mRNA expression in both Groups 2 and 4 remained significantly above the control levels through all time-points. However, when the progression of type-III collagen expression was examined through each time-point, Group 2 (tendon injury) exhibited a significant decrease in expression at three weeks that was more than twofold less than the expression at both one and five weeks. Yamakawa et al.⁴⁴ and Kobayashi et al.⁴⁵ both examined type-III collagen expression following an acute incisional injury to tendons in an avian model that are considered to be equivalent to the human rotator cuff. Neither

study noted a decrease in type-III expression at three weeks. Yamakawa et al. reported maximum mRNA expression at two weeks after the injury, whereas Kobayashi et al. demonstrated early expression at two weeks followed by a progressive increase to maximum expression at six weeks. Perry et al., however, recently demonstrated a similar decrease in the expression of an inflammatory factor in an overuse injury of the supraspinatus tendon in a rat model⁴⁶. Following an initial increase in the expression of 5-lipoxygenase activating protein, a marker for the lipoxygenase inflammatory pathway, a decrease in the mRNA level was noted between one and two weeks. Expression then increased dramatically to maximum levels at eight weeks. Despite a similar expression trend following rotator cuff tendon injury, it is still difficult to compare data from an overuse, chronic model with our incisional, acute injury model. Nonetheless, their findings illustrate that the expression of factors in response to tendon injury does not necessarily follow a progressively increasing course toward a maximum expression level. Although type-III collagen is not considered an inflammatory factor, it would not be unreasonable to consider it a part of the acute phase response given its early expression following tendon injury. Given the similar time frame of inflammatory factor activation and type-III collagen expression, some interaction between the two tendon injury responses would be expected. Interestingly, Group 4 (tendon injury and steroid treatment) did not demonstrate a significant decrease in type-III collagen expression at three weeks and levels remained 1.5-fold higher ($p < 0.05$) than that in Group 2 (tendon injury). This would suggest that corticosteroid modulates type-III collagen expression and maintains the level at one week through the third week. Whether the mechanism may be related to the inhibitory effect of the corticosteroid on the inflammatory arachidonic acid cascade remains to be determined⁴⁷⁻⁴⁹.

Type-III to Type-I Collagen Expression Ratio

The type-III to type-I collagen expression ratio was calculated for each individual tendon in all groups. It is our impression from these data that the expression ratio is the most important value when evaluating the injury response of tendons. When tendons are injured, an increased proportion of type-III collagen is produced during the early reparative response. As injured tendons remodel and mature, baseline type-III to type-I collagen expression ratios are restored. Collagen protein typing of injured tendons has shown that type-III collagen can increase up to 15% of total collagen content following injury⁴³. Collagen fibers have been found to be not only smaller in diameter but also structurally weaker to tensional forces when type-III content is higher^{43,50,51}. Consequently, the type-III to type-I collagen expression ratio is an ideal dynamic indicator of tendon injury response and also provides an important insight into the functional strength and resiliency of the tendon.

Comparison of the type-III to type-I collagen expression ratios in Groups 2 (tendon injury) and 4 (tendon injury and steroid treatment) suggests that a single dose of methylprednisolone equivalent to that given to humans does not significantly alter the acute phase response of an injured rotator cuff tendon. In both groups, a significant increase in the expression of type-III collagen over type-I collagen was visible at one week. The two groups then showed an equal, progressive decline in the type-III to type-I collagen expression ratio until it was no longer significantly above the control level at five weeks.

Interestingly, exposure of the uninjured tendons in Group 3 (steroid treatment) to the same methylprednisolone dose resulted in a significant increase in the type-III to type-I collagen expression ratio. The magnitude of this response was equivalent to the injury response seen in Group 2 (tendon injury). This significant response to steroid in normal tendon, however, was short-lived, and the type-III to type-I collagen expression ratio decreased to control levels by the third week.

To our knowledge, only two published animal studies have specifically examined the possible adverse effects of sub-acromial corticosteroid injections on rotator cuff tendons. Both studies used a rat shoulder model, which has been established as a reliable *in vivo* model for studying rotator cuff abnormalities^{22–24}. Tillander et al. evaluated the effects of repeated subacromial corticosteroid injections by injecting rats with either three or five doses of triamcinolone in a dosage equivalent to that given to humans spaced at one-week intervals²⁶. The infraspinatus and supraspinatus tendons were then evaluated macroscopically and histologically. The tendons of both the control group, which received injections of saline solution, and the group that received three steroid injections showed no evidence of any pathologic change. After five repeated injections, however, the rotator cuff tendons exhibited clear morphologic changes, becoming whiter and less smooth, and, histologically, the tendons developed signs of necrosis and collagen bundle fragmentation. Inflammatory cells were also evident in high numbers between collagen bundles. Akpinar et al. examined the adverse effects of repeated injections of methylprednisolone and betamethasone, both at a dosage equivalent to that given to humans²⁵. Subacromial injections were given at two-week intervals for a total of eight weeks. The infraspinatus and supraspinatus tendons of both corticosteroid-treated groups were found to be softer and lighter in color than the tendons exposed to saline solution. Histologic changes, including evidence of necrosis, collagen-bundle fragmentation, and infiltration of inflammatory cells, were also present at eight weeks following repeated subacromial corticosteroid exposure. No significant differences, however, were found between the tendons of the two different corticosteroid groups.

These two studies clearly demonstrate the detriment of multiple subacromial corticosteroid injections given at short intervals to rotator cuff tendons. Yet as Tillander et al. demonstrated, a series of three injections at one-week intervals did not result in pathologic changes on morphologic or histologic examination²⁶. While this may suggest that the adverse effects of corticosteroids are additive and significant only after reaching a certain threshold, it may also be that the early effects are simply not detectable at macroscopic and histologic levels. Additionally, both studies analyzed the effect of multiple corticosteroid doses on healthy, uninjured rotator cuff tendons. This does not necessarily reflect clinical applications of corticosteroid injections given at limited intervals in the presence of a symptomatic rotator cuff abnormality.

The acute injury in a rat model used in this study does not duplicate the clinical settings, in which subacromial corticosteroid injections are most often used. However, there are no well-established and acceptable animal models for reproducing chronic rotator cuff tendinosis and partial-thickness tears at this time. Our emphasis was to clarify the largely unexamined interplay between injured rotator cuff tendons and corticosteroids rather than to differentiate the type and chronicity of rotator cuff tendon injury. Additionally, the information gained from this acute injury model may provide insight into chronic injury responses. Investigators have demonstrated that elevated levels of type-III collagen are present in chronic tendinopathies and degenerative tendon tears as well^{30,43,50}.

In conclusion, the findings in this study suggest that a single dose of methylprednisolone has no lasting effect on the collagen expression of either injured or uninjured rat rotator cuff tendons through five weeks. The acute injury response of rotator cuff tendons appears largely unaffected by a single dose of methylprednisolone. At the same time, the significant injury response seen in the group that had steroid treatment only suggests that a single dose might not be entirely benign, even to healthy rotator cuff tendons, during the first few weeks following injection. The dramatic increase in the type-III to type-I collagen expression ratio can have an appreciable effect on the biomechanical properties of rotator cuff tendons if the same proportions of collagen type are translated to the protein level. As a result, clinically, it

might be prudent to avoid aggressive shoulder motion and strengthening programs within the first two to three weeks following a subacromial corticosteroid injection.

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Fig. 1. Illustration demonstrating the rat forelimb. The supraspinatus and infraspinatus tendons run under an osseous acromial arch (inset). The surgical defect in the infraspinatus tendon spans 50% of the tendon width, 5 mm proximal to the insertion site.

Fig. 2.
The type-III to type-I collagen expression ratio.

TABLE I

Gene Expression of Type-I Collagen

Week	Type-I Collagen mRNA ^{*†} (percent)		
	Group 2 (Tendon Injury)	Group 3 (Steroid Treatment)	Group 4 (Tendon Injury and Steroid Treatment)
1	105 ^{a,1}	63 ^{a,3}	98 ^{a,5}
3	84 ^{b,1}	160 ^{c,3}	167 ^{c,5}
5 [‡]	288 ^{d,2}	298 ^{d,4}	398 ^{d,6}

* The percentages indicate the amount relative to the value for the respective control group.

[†] The letters in superscript indicate a comparison in rows (between groups), and the numbers in superscript indicate a comparison in columns (between weeks). When the data points have the same letter or number, the difference is not significant. When the data points have a different letter or number, the difference is significant ($p < 0.05$).

[‡] The values at five weeks for all three groups are significantly different from the control value ($p < 0.05$).

TABLE II

Gene Expression of Type-III Collagen

Week	Type III-Collagen mRNA ^{*†} (percent)		
	Group 2 (Tendon Injury)	Group 3 (Steroid Treatment)	Group 4 (Tendon Injury and Steroid Treatment)
1	578 ^{a,1}	426 ^{a,3}	598 ^{a,4,5}
3	252 ^{b,2}	300 ^{b,c,3}	402 ^{c,4}
5	514 ^{d,e,1}	426 ^{d,3}	698 ^{e,5}

* The percentages indicate the amount relative to the value for the respective control group. All values were significantly greater than the control values ($p < 0.05$).

† The letters in superscript indicate a comparison in rows (between groups), and the numbers in superscript indicate a comparison in columns (between weeks). When the data points have the same letter or number, the difference is not significant. When the data points have a different letter or number, the difference is significant ($p < 0.05$).