

# Factors Expressed in an Animal Model of Anteroinferior Glenohumeral Instability

Mary K. Mulcahey,<sup>\*†</sup> MD, Mindy Marshall,<sup>‡</sup> Stacey E. Gallacher,<sup>§</sup> MD, Lee A. Kaback,<sup>||</sup> MD, and Theodore A. Blaine,<sup>§</sup> MD

*Investigation performed at the Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA*

**Background:** There is little information on the molecular factors important in healing and changes that occur in the glenoid labrum in response to injury. Using a novel animal model of acute anterior shoulder dislocation, this study characterizes the factors expressed in the glenoid labrum in response to injury and correlates their expression to glenohumeral stability.

**Purpose:** To study the response of the glenoid labrum to injury both biomechanically and with immunohistochemical testing.

**Methods:** An injury to the anteroinferior labrum was surgically induced in 50 male Lewis rats. Rats were sacrificed at 3, 7, 14, 28, or 42 days. Immunolocalization experiments were performed to localize the expression of growth factors and cytokines. For biomechanical testing, dynamic stiffness for anterior and posterior laxity, load to failure, stiffness, and maximum load were recorded. Statistical differences were determined at  $P < .05$ .

**Study Design:** Descriptive laboratory study.

**Results:** Expression of interleukin-1 beta (IL-1 $\beta$ ), transforming growth factor-beta 1 (TGF- $\beta$ 1), matrix metalloproteinase 3 (MMP3), and matrix metalloproteinase 13 (MMP13) were increased in injured compared with uninjured specimens. Collagen III expression was increased early and decreased with time. Biomechanical testing verified instability by demonstrating increased anterior displacement and decreased stiffness in injured shoulders at all time points.

**Conclusion:** This novel animal model of acute anterior shoulder dislocation showed increased expression of IL-1 $\beta$ , TGF- $\beta$ 1, MMP3, MMP13, and collagen III in the injured labral tissue at early time points. Increased anterior laxity and decreased stiffness and maximum load to failure were seen after anterior labral injury, supporting the model's ability to re-create anterior glenohumeral instability. These data provide important information on the temporal changes occurring in a rat model of anterior glenohumeral dislocation.

**Clinical Relevance:** Identification of factors expressed in the anterior capsule and glenoid labrum in response to injury may lead to the development of novel agents that can be used to augment glenoid labrum healing and ultimately improve both surgical and nonsurgical treatment of this common shoulder injury.

**Keywords:** animal model; shoulder instability; anterior shoulder dislocation; glenoid labrum; growth factors; cytokines; biomechanical testing

Shoulder dislocation is a common injury and most often occurs in the anteroinferior direction. During injury, the displaced humeral head stretches the capsuloligamentous

structures, causing capsular tear<sup>4,28,35</sup> and/or labral avulsion.<sup>5,6,11,17,18,25,35,36</sup> Anteroinferior labral lesions often require surgical fixation due to persistent anterior glenohumeral instability.<sup>3,13,19,26</sup> Despite the common nature of acute anterior glenohumeral dislocation, there is little information on the biologic and biochemical factors that are important in healing of the glenoid labrum.

Bankart was the first to characterize the anatomic pathology of shoulder instability, calling the anteroinferior labral tear “the essential defect” in shoulder instability.<sup>16</sup> The glenoid labrum is composed of fibrocartilaginous tissue at the junction of the capsule of the shoulder and the glenoid.<sup>27</sup> Because of the absence of information available on glenoid labral healing, most information must be extrapolated from our knowledge of the knee meniscus. The glenoid labrum is similar to the meniscus in the knee in that only the peripheral margin has a significant vascular supply.<sup>1,10</sup>

\*Address correspondence to Mary K. Mulcahey, MD, 245 North 15th Street, Mail Stop #420, Philadelphia, PA 19102, USA (email: mary.mulcahey.md@gmail.com).

<sup>†</sup>Drexel University College of Medicine, Philadelphia, Pennsylvania, USA.

<sup>‡</sup>EpiVax Inc, Providence, Rhode Island, USA.

<sup>§</sup>Yale University School of Medicine, New Haven, Connecticut, USA.

<sup>||</sup>OrthoNY, Albany, New York, USA.

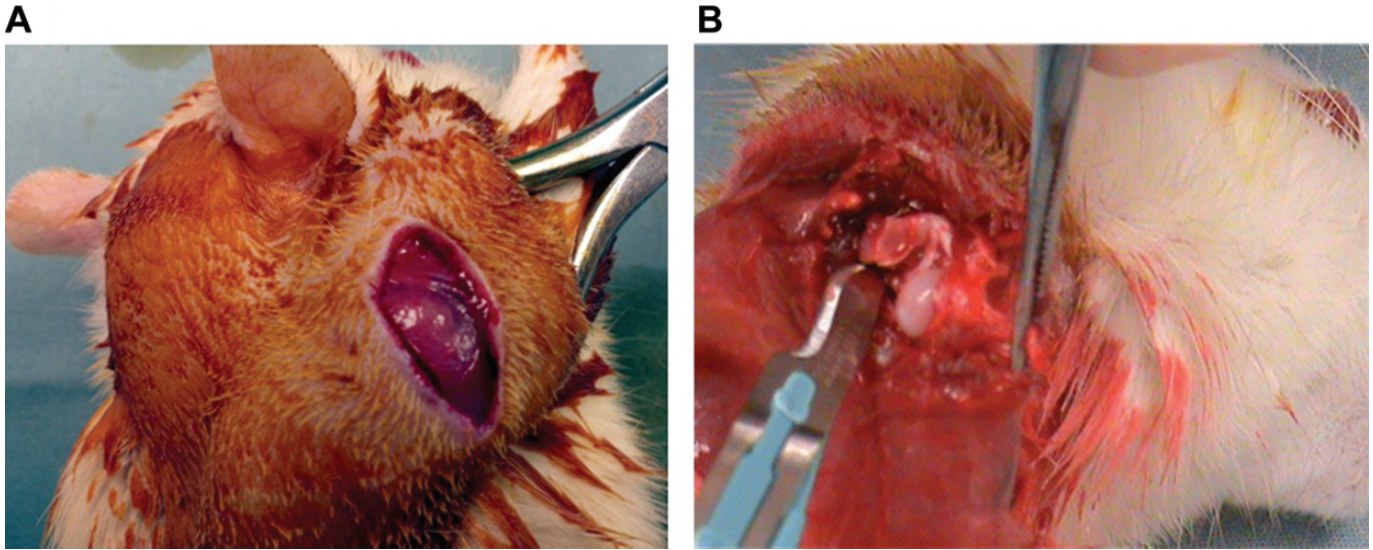
One or more of the authors has declared the following potential conflict of interest or source of funding: M.K.M and T.A.B. received funding through an Orthopaedic Research and Educational Foundation Grant.

The Orthopaedic Journal of Sports Medicine, 3(8), 2325967115599733

DOI: 10.1177/2325967115599733

© The Author(s) 2015

This open-access article is published and distributed under the Creative Commons Attribution - NonCommercial - No Derivatives License (<http://creativecommons.org/licenses/by-nc-nd/3.0/>), which permits the noncommercial use, distribution, and reproduction of the article in any medium, provided the original author and source are credited. You may not alter, transform, or build upon this article without the permission of the Author(s). For reprints and permission queries, please visit SAGE's Web site at <http://www.sagepub.com/journalsPermissions.nav>.



**Figure 1.** Glenoid labrum tear in a rat model. (A) Posterior approach to the glenohumeral joint. (B) Elevation of the anteroinferior labrum off of the glenoid using a No. 15 scalpel. The scalpel is along the anteroinferior glenoid seen centrally with the distracted humeral head seen to the right.

The inner, avascular third of the meniscus has limited healing capacity while the outer two-thirds may heal by scar formation.<sup>2</sup> Soluble mediators (eg, growth factors and cytokines) or physical factors (eg, mechanical compression or stretch) can have a significant influence on the metabolic activity of meniscal cells.<sup>15</sup>

Some of the important factors in meniscal healing include transforming growth factor- $\beta$  (TGF- $\beta$ ) and interleukin-1 (IL-1). Transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) stimulates mesenchymal cells to produce specific proteoglycans and type II collagen.<sup>34</sup> The expression of proinflammatory cytokines such as IL-1 is known to be elevated in the presence of joint injury in the knee.<sup>14,21,37</sup>

This study was designed to develop an animal model of glenohumeral instability and to determine the immunohistochemical and mechanical changes that occur in the labrum in response to injury. Unlike the meniscus, there is currently no reliable animal model for glenoid labral injuries, and little is known about its capacity for healing. Soslowsky et al<sup>33</sup> demonstrated that the bony, ligamentous, and muscular anatomy of the rat shoulder resembles that of the human, making it a good model to study shoulder pathology.<sup>8,30</sup> These protocols are well established and applicable to the experimental design of this study as well.<sup>12,33</sup> Based on studies of proinflammatory cytokines and growth factors in the meniscus, we hypothesized that, similarly, IL-1 $\beta$ , matrix metalloproteinases 3 and 13 (MMP3 and MMP13), TGF- $\beta$ 1, and collagen type III will be induced in the healing glenoid labrum and capsule in response to injury. This study also performed biomechanical testing to verify the ability of the model to create glenohumeral instability and to correlate the changes in growth factors and cytokines with biomechanical changes that occur.

## METHODS

### Glenoid Labral Injury

Eight-week-old male Lewis rats weighing approximately 250 g (N = 50) were used in this study. This species was recommended by our Institutional Animal Care and Use Committee (IACUC), and rats of sufficient size and weight were required to perform biomechanical testing and histology. Ten rats were used for immunohistochemical analysis, and 40 rats were used for biomechanical analysis. The studies were approved by our hospital's IACUC (biomechanical study, CMTT 0064-11; immunohistochemistry study, CMTT 0092-10).

The male Lewis rats were anesthetized with an intraperitoneal injection of Dexdomitor (Zoetis; 0.5 mg/kg)/ketamine (75 mg/kg) at the time of surgery, followed by an intramuscular dose of cefazolin (Ancef; SmithKline; 20 mg/kg). Once the animals were anesthetized, the posterior aspect of the right shoulder was shaved and prepped, and the surgical field was sterilely draped. A 3- to 4-cm incision was made longitudinally along the posterior aspect of the shoulder. The deltoid was incised posteriorly to expose the posterior rotator cuff, and the rotator cuff and capsule were incised longitudinally to expose the glenohumeral joint. Traction was applied to the forelimb to expose the glenoid and the attached labrum. A No. 15 scalpel was used to create a small laceration in the anteroinferior labrum at the level of the glenoid rim (Figure 1). The laceration included the entire anterior glenoid labrum to include the entire anteroinferior glenohumeral ligament and continued inferiorly to a point just past the 6 o'clock position on the glenoid face. The shoulder was then traumatically dislocated anteriorly and inferiorly until the humeral head was completely dislocated on the glenoid. The shoulder was

allowed to relocate, and the posterior capsulotomy and rotator cuff defect were repaired using absorbable sutures. No repair was performed anteriorly. The labral lesion was created prior to dislocation to ensure that a Bankart lesion was created in the same location rather than instability occurring from other pathology or injury to the remaining capsule. The subcutaneous tissue was closed with 4-0 absorbable sutures, and the skin was closed using wound clips. This procedure was performed by the same 2 investigators (M.K.M. and S.E.G.) and was easily reproducible. The same procedure was used for each rat shoulder, and quantification of the size of the laceration was performed by visual inspection by the surgeon as described.

The effects of anesthesia were reversed using atipamezole hydrochloride (Antisedan; Zoetis; 1.0 mg/kg) given by intramuscular injection. The animals were placed in separate cages and kept warm while recovering from surgery. Once fully awake, the animals received buprenorphine hydrochloride (0.03 mg/kg) subcutaneously. Buprenorphine 0.03 mg/kg subcutaneously was also given twice daily for the first 2 postoperative days. Animals were allowed to move freely in the cage, and no immobilization was used. Most animals avoided using the affected limb for the first several days. After the first 2 to 3 postoperative days, the animals showed no obvious signs of limb dysfunction or gross instability. The clinical results were not recorded and were not correlated with immunohistochemical or biomechanical testing.

#### Immunohistochemical Staining of Rat Labrum

Immunolocalization experiments were performed to localize the expression of growth factors and cytokines (TGF- $\beta$ 1, IL-1 $\beta$ , MMP3, MMP13, and collagen III). Ten rats, divided into groups of 2, were sacrificed by carbon dioxide inhalation at 3, 7, 14, 28, or 42 days. Both the injured and contralateral uninjured shoulders were harvested and prepared for histological and immunohistochemical analysis. The glenohumeral joint was isolated and included the bony and capsulolabral tissue for histological analysis. The surrounding tissues including the muscle and rotator cuff were not included in the preparation. Tissues were harvested and preserved in 4% paraformaldehyde. Tissues were then decalcified and embedded in paraffin blocks, and 5- $\mu$ m coronal sections incorporating the injured labrum of each shoulder were cut. Sections were deparaffinized by sequential immersion in a series of graded xylene and ethanol. Endogenous peroxidase activity was quenched, and blocking serum was applied. Sections were incubated with primary and secondary antibodies of relevant genes, followed by conjugation of horseradish peroxidase to secondary antibodies. Chromogenic reaction occurred by immersing sections in 0.05% diaminobenzidine tetrahydrochloride. Counterstaining was done using hematoxylin-eosin or methyl green.

#### Grading Slides

Histologic analysis was performed semiquantitatively using a grading scale, where 0 indicated <5% staining, 1 indicated  $\geq$ 20% staining, 2 indicated  $\geq$ 50% staining, and

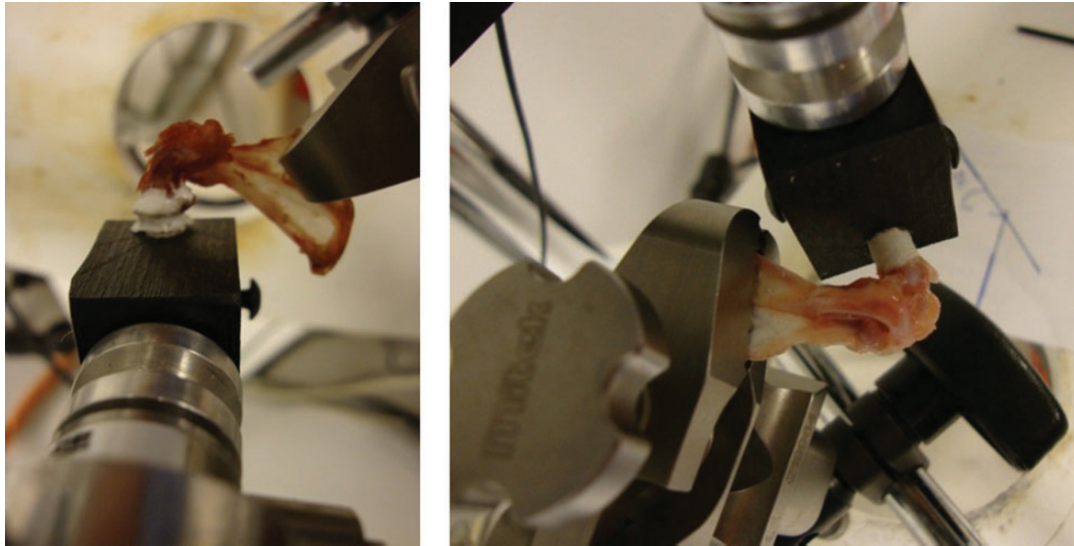
3 indicated  $\geq$ 90% staining. This analysis was performed independently by 3 graders blinded to specimen group. The fibrocartilaginous tissue immediately adjacent to the glenoid articular cartilage was identified as the labral tissue and area of focus for histologic analysis. The surrounding tissues including muscle and rotator cuff were not included in the preparation. All 3 graders were instructed to examine these sections when grading slides.

#### Biomechanical Testing of Rat Labrum

Forty rats, divided into groups of 10, were sacrificed by carbon dioxide inhalation at 3, 7, 14, or 28 days. After sacrifice, the forelimbs were removed and dissected to isolate the glenohumeral joint capsule, humerus, and scapula. The humeri were potted in urethane (Smooth-On) with the assistance of a custom alignment fixture and were mounted to the actuator of an EnduraTec ELF 3200 load frame (EnduraTec Systems) using custom pull-out fixtures. The scapulae were clamped rigidly using custom fixtures in the same horizontal plane such that there was no internal or external rotation. The actuator moved up and down simulating anterior and posterior displacement of the humerus with respect to the glenoid. The glenohumeral joint was fixed at 45° of abduction, neutral rotation (Figure 2). Anterior-to-posterior laxity fatigue was performed for 100 cycles each at a frequency of 1 Hz and a load of 3.64% of the weight of the rat at the time of sacrifice. Glenohumeral laxity research in human cadavers has commonly used  $\pm$ 25 N as the metric to analyze laxity differences between repair techniques.<sup>24,39</sup> We theorized that a 25-N force in a 70-kg human equated to 3.64% of the mean force of gravity. Monotonic loading in anterior tension at a constant displacement rate of 0.25 mm/s was completed on conclusion of fatigue testing until failure. Load and displacement data were acquired using WinTest collection software (EnduraTec Systems). Dynamic stiffness for anterior and posterior laxity, load to failure, stiffness, and maximum load were recorded. The uninjured shoulder was used as a control.

#### Statistical Analysis

Because of the small sample size for immunohistochemical staining, no statistical analysis was able to be performed. Generalized estimating equations were used to compare injured and uninjured limbs at different days postinjury. Within-animal limb differences were modeled as having a heterogeneous compound symmetric variance covariance structure (each limb has a variance and a covariance between limbs), block diagonal by day of sacrifice. Laxity measures and yield load were positively skewed and modeled using a lognormal distribution. Maximum load and displacements were modeled as Gaussian. Comparisons between limbs at each day of sacrifice were maintained at an alpha of 0.05 using the Holm test to adjust each *P* value. Sandwich estimation was used to adjust for any model misspecification. Differences between injured and uninjured limbs were expressed as a ratio using 95% confidence limits.



**Figure 2.** Apparatus for biomechanical testing of injured and noninjured rat shoulders. The humerus is potted in the apparatus, and the scapula is seen clamped.

## RESULTS

Hematoxylin-eosin staining of the capsulolabral injury group and noninjured controls at 3 and 7 days (Figure 3) demonstrated widening of the glenohumeral joint and disruption of the glenoid labrum versus uninjured controls (Figure 3, A and B). At higher magnification, the injured specimen displayed marked hypercellularity at 7 days compared with the uninjured labrum (Figure 3, C and D). There was no obvious degeneration noted in the articular cartilage of the visible joint surfaces on histologic examination.

While the sample size for each factor tested was small, trends in expression and localization were able to be observed. Expression of the proinflammatory cytokine IL-1 $\beta$  was increased at all time periods in injured tissue compared with uninjured tissue (Figure 4). Expression of TGF- $\beta$ 1 was also increased in injured compared with uninjured tissue at 3, 14, and 42 days (Figure 4). Expression was diffusely localized and present both in cells and surrounding fibrous tissue (Figure 5). MMP3 and MMP13 were both expressed at increased levels in injured tissue compared with uninjured tissue at 3, 7, and 14 days, although there was baseline expression of MMP3 at all time points in uninjured controls (data not shown). MMP expression was primarily localized to inflammatory cells and fibroblasts (Figure 5). Collagen III expression was also increased early at 3 days and gradually decreased with time by 42 days postinjury (Figure 4). Expression was diffusely localized throughout the injured tissue.

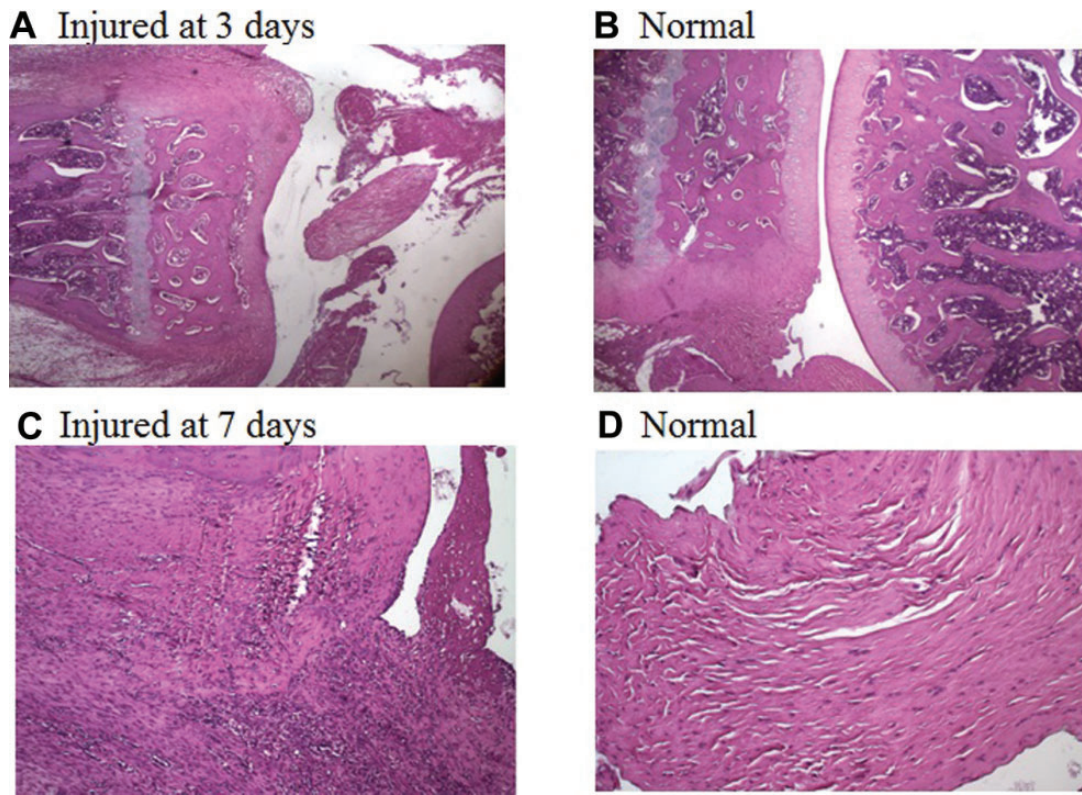
### Biomechanical Analysis

Clinically, the rats did not demonstrate any obvious impairment in function or gross instability postoperatively; therefore, clinical outcome was not correlated with biomechanical testing. Biomechanical testing verified the presence

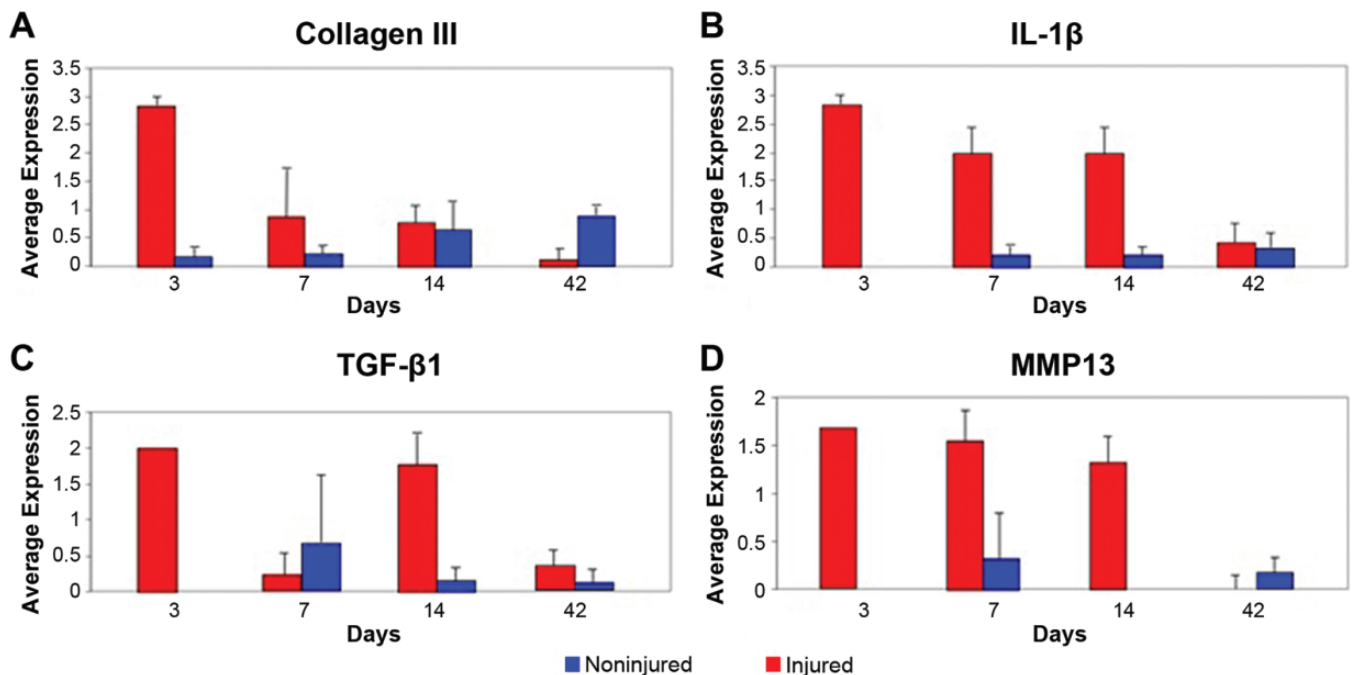
of instability created by this model. Injured shoulders demonstrated increased anterior laxity at all time points compared with controls (Figure 6), although statistically significant differences occurred only at day 7 ( $P = .0001$ ) and day 14 ( $P = .0434$ ). The amount of displacement increased over time in injured shoulders, whereas it did not significantly change in the control group. At 2 weeks, injured and uninjured shoulders displayed similar maximum load characteristics (Figure 7A). At 3, 7, and 28 days, the injured shoulders were able to withstand lower levels of maximum load compared with controls (Figure 7A), and this difference was statistically significant at the day 3 time point ( $P = .0008$ ). The continued presence of decreased maximum load in injured shoulders at 28 days suggests an inadequate healing response to injury. Displacement to yield was increased in injured compared with noninjured specimens at 3, 7, and 14 days, although these differences were not statistically significant (data not shown). Injured shoulders demonstrated decreased stiffness compared with uninjured shoulders at all time points (Figure 7B), with statistically significant decreases noted at 7 ( $P = .0191$ ) and 28 days ( $P = .0362$ ). These findings support the ability of this model to re-create anterior glenohumeral instability.

## DISCUSSION

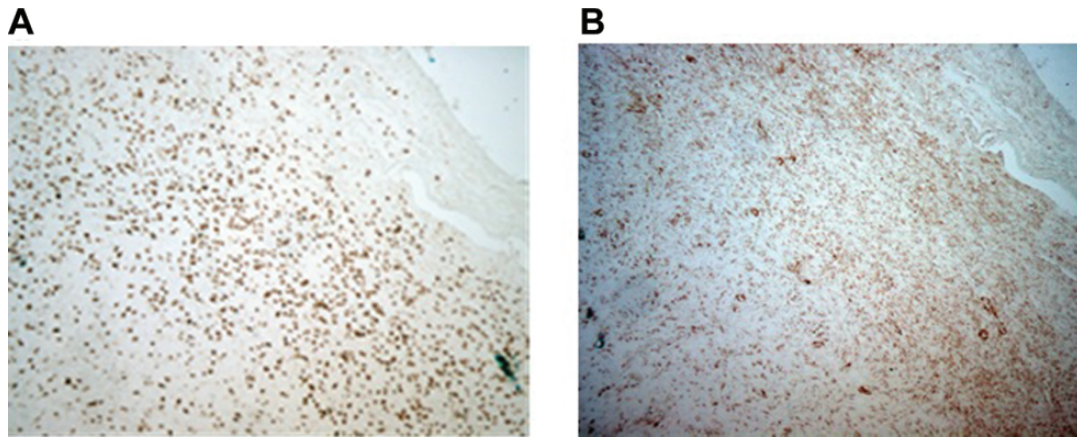
In this novel animal model of acute anteroinferior labral injury with resultant anteroinferior glenohumeral instability, we found increased expression of proinflammatory cytokines IL-1 $\beta$ , TGF- $\beta$ 1, MMP3, and MMP13 in the injured labral tissue when compared with uninjured control shoulders. Expression of these factors occurred early at 3 days following injury and decreased by 42 days postinjury. While TGF- $\beta$ 1 was diffusely expressed, MMP and IL-1 $\beta$  expression were primarily localized to inflammatory and fibroblastic cells.



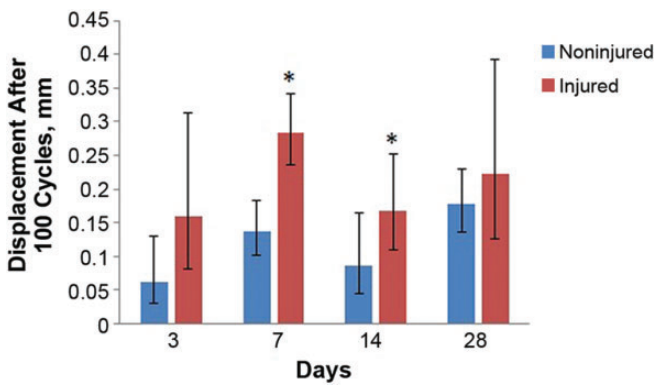
**Figure 3.** Hematoxylin-eosin staining of injured and noninjured rat labrum at (A, B) 3 days and (C, D) 7 days. (A, B) Magnification at 4× demonstrating experimental glenoid labral injury at 3 days postinjury. Displacement of the humeral head from the glenoid is evident in (A) injured compared with (B) noninjured controls. (C, D) Magnification at 20× demonstrating experimental glenoid labral injury at 7 days postinjury demonstrating hypercellularity in the (C) injured labrum and capsule compared with (D) the uninjured control.



**Figure 4.** Immunohistochemical staining grades comparing injured (red) versus noninjured (blue) rat labral tissue. (A) Collagen type III, (B) Interleukin-1 beta (IL-1β), (C) transforming growth factor-beta 1 (TGF-β1), (D) matrix metalloproteinase 13 (MMP13).



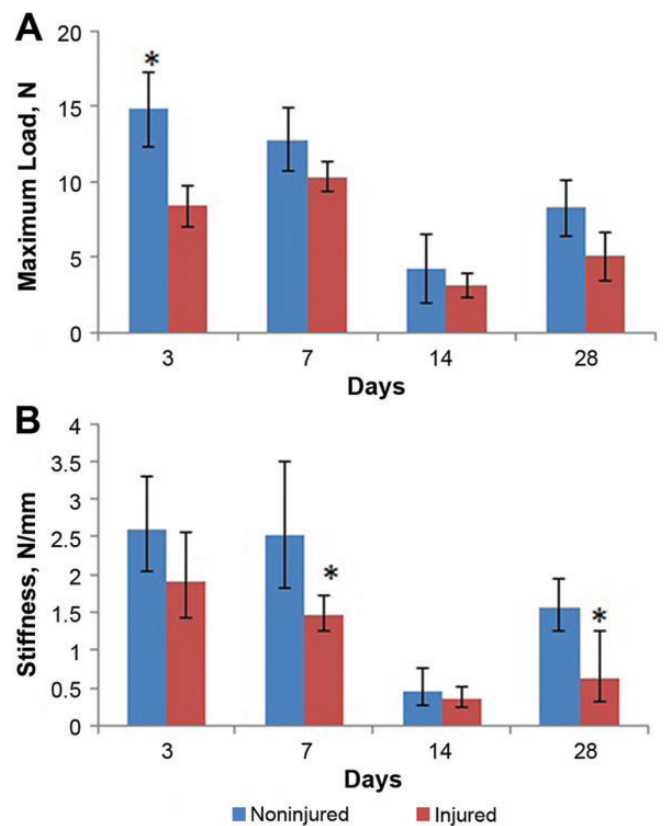
**Figure 5.** Immunohistochemistry photomicrographs of injured labrum 7 days postinjury. (A) Matrix metalloproteinase 13 (MMP13) (localized within cells) and (B) transforming growth factor-beta 1 (TGF-beta1) (diffuse expression).



**Figure 6.** Biomechanical stability testing comparing injured and noninjured rat glenohumeral joint tissue. \*Statistically significant.

There are no prior investigations on the role of cytokines in labral healing; however, the meniscus in the knee may be analogous to healing in the glenoid labrum. A recent *in vitro* study by Hennerbichler et al<sup>15</sup> revealed treatment of meniscal tissue with either IL-1 or tumor necrosis factor-alpha (TNF- $\alpha$ ) inhibited cell accumulation and meniscal repair. Elevated cytokines such as IL-1 and TNF in the injured labrum may therefore lead to suppression of matrix biosynthesis and increased enzymatic degradation.<sup>15</sup>

In this study, we found consistent elevation of IL-1 $\beta$  in the injured glenoid labrum as compared with controls. Increased levels of IL-1 stimulate catabolic pathways and inhibit anabolic pathways in joint tissues. IL-1 expression leads to increased production of inflammatory mediators, including prostaglandin E2 (PGE2) and nitric oxide (NO), stimulates transcription and activity of MMP, increases release of proteoglycans, and inhibits collagen and aggrecan synthesis.<sup>38</sup> Therefore, the increased expression of IL-1 $\beta$  that we observed in the injured glenoid labrum may play a role in an inadequate healing response that occurs in the glenoid labrum after shoulder dislocation.



**Figure 7.** Biomechanical testing comparing (A) maximum load and (B) stiffness of injured and noninjured rat glenohumeral joint tissue. \*Statistically significant.

We also observed increased expression of TGF- $\beta$ 1 in the injured glenoid labrum as compared with control specimens at 3, 14, and 42 days postinjury. Several animal models have demonstrated an association of TGF- $\beta$ 1 with scar and adhesion formation.<sup>9,23,29</sup> TGF- $\beta$ 1 and TGF- $\beta$ 3 play an important role in regulating musculoskeletal growth and

differentiation.<sup>22,29</sup> Adult wound healing in skin and tendon midsubstance occurs with scar formation and elevated levels of TGF- $\beta$ 1 through a process of remodeling.<sup>29,31,32</sup> The expression of TGF- $\beta$ 1 in injured labral tissue is consistent with these prior data and supports inadequate healing by scar tissue formation as a result of labral injury.

We also observed increases in collagen type III in the injured labrum after shoulder dislocation. Increased levels of collagen type III have been seen in degenerated and ruptured tendons.<sup>7,20</sup> Galatz et al<sup>12</sup> used a rat model to investigate collagen formation at the early repair site in rotator cuff healing. Immunohistochemical results demonstrated disorganized scar material present at the insertion site and expression of type III collagen.<sup>12</sup> Our immunohistochemical analysis revealed similar results, showing that collagen III expression was diffusely expressed early following injury, with a gradual decrease at later time points. Increased expression of collagen III in the glenoid labrum further supports scar formation in response to labral injury. The immunohistochemical findings in this study are limited to the capsulolabral tissue, and the surrounding tissue response to injury in the rotator cuff muscles and tendons was not evaluated.

Biomechanical analysis of rat shoulders with acute anterior instability revealed increased anterior laxity as compared with uninjured shoulders. Laxity increased by 4 weeks, suggesting persistent instability and labral injury in these shoulders. Stiffness and maximum load to failure also decreased in injured compared with uninjured shoulders, further supporting persistent instability. In a similar study of rat rotator cuff injury, Galatz et al<sup>12</sup> found a gradual improvement in biomechanical properties over time. In contrast, the biomechanical results from our study did not support any improvement in laxity by the 28-day time point. This suggests that although the rotator cuff is capable of healing to a certain extent with scar tissue, the glenoid labrum may not be capable of such a response.

There are limitations to this study. The animal model used in this study requires an acute laceration of the anterior labrum and does not directly correlate to the traumatic injury that occurs in humans. To control for this discrepancy, we used a combined model of injury where an acute labral laceration/simulated tear was followed by a traumatic dislocation where the humeral head was forcefully dislocated in an anterior-inferior direction. We feel that this mechanism more closely represents the injury that occurs in vivo. In addition, a sham control was not performed in this study; therefore, some of the immunohistochemical changes seen may be due to the surgery alone or to the global injury that occurs during a dislocation rather than specifically a labral tear. Additional studies will assess the effect of the surgical approach on these findings. Third, only a small subset of growth factors known to be involved with impaired meniscus and tendon healing were examined in this study. Many other growth factors and cytokines are involved with this healing process and likely contribute to the inadequate healing response seen following glenoid labral injury. Further studies are planned to determine the role of these and other factors and their relationships to glenoid labral healing.

## CONCLUSION

There is currently no reliable animal model for glenohumeral instability, and little is known about the capacity for glenoid labral healing after shoulder dislocation. Increased anterior laxity and decreased stiffness and maximum load to failure were seen after anterior labral injury, supporting the model's ability to re-create anteroinferior glenohumeral instability. In this novel animal model, we found increased expression of cytokines, growth factors (IL-1 $\beta$ , TGF- $\beta$ 1, MMP3, and MMP13), and collagen III in the injured labral tissue. These immunohistochemical and biomechanical data provide important information on the temporal changes that occur after anterior glenohumeral dislocation and anterior labral injury. Identification of these and other factors may lead to the development of novel agents that can be used to augment glenoid labrum healing and ultimately improve both surgical and nonsurgical treatment of this common shoulder injury.

## REFERENCES

1. Arnoczky SP, Warren RF. Microvasculature of the human meniscus. *Am J Sports Med.* 1982;10:90-95.
2. Arnoczky SP, Warren RF. The microvasculature of the meniscus and its response to injury. An experimental study in the dog. *Am J Sports Med.* 1983;11:131-141.
3. Altchek DW, Warren RF, Wickiewicz TL, Ortiz G. Arthroscopic labral debridement. A three-year follow-up study. *Am J Sports Med.* 1992; 20:702-706.
4. Baker CL, Uribe JW, Whitman C. Arthroscopic evaluation of acute initial anterior shoulder dislocations. *Am J Sports Med.* 1990;18:25-28.
5. Bigliani LU, Kurzweil PR, Schwartzbach CC, Wolfe IN, Flatow EL. Inferior capsular shift procedure for anterior-inferior shoulder instability in athletes. *Am J Sports Med.* 1994;22:578-584.
6. Bottoni CR, Wickens JH, DeBerardino TM, et al. A prospective, randomized evaluation of arthroscopic stabilization versus nonoperative treatment in patients with acute, traumatic, first-time shoulder dislocations. *Am J Sports Med.* 2002;30:576-580.
7. Boyer MI, Harwood F, Ditsios K, Amiel D, Gelberman RH, Silva MJ. Two-portal repair of canine flexor tendon insertion site injuries: histologic and immunohistochemical characterization of healing during the early postoperative period. *J Hand Surg Am.* 2003;28:469-474.
8. Carpenter JE, Thomopoulos S, Flanagan CL, DeBano CM, Soslowky LJ. Rotator cuff defect healing: a biomechanical and histologic analysis in an animal model. *J Shoulder Elbow Surg.* 1998;7:599-605.
9. Chang J, Thunder R, Most D, Longaker MT, Lineaweaver WC. Studies in flexor tendon wound healing: neutralizing antibody to TGF- $\beta$ 1 increases postoperative range of motion. *Plast Reconstr Surg.* 2000;105:148-155.
10. Cooper DE, Arnoczky SP, O'Brien SJ, Warren RF, DiCarlo E, Allen AA. Anatomy, histology, and vascularity of the glenoid labrum. An anatomical study. *J Bone Joint Surg Am.* 1992;74:46-52.
11. Coughlin L., Rubinovich M, Johansson J, White B, Greenspoon J. Arthroscopic staple capsulorrhaphy for anterior shoulder instability. *Am J Sports Med.* 1992;20:253-256.
12. Galatz LM, Sandell LJ, Rothermich SY, et al. Characteristics of the rat supraspinatus tendon during tendon-to-bone healing after acute injury. *J Orthop Res.* 2006;24:541-550.
13. Glasgow SG, Bruce RA, Yacoubucci GN, Torg JS. Arthroscopic resection of glenoid labral tears in the athlete: a report of 29 cases. *Arthroscopy.* 1992;8:48-54.
14. Hashimoto S, Takahashi K, Ochs RL, Coutts RD, Amiel D, Lotz M. Nitric oxide production and apoptosis in cells of the meniscus during experimental osteoarthritis. *Arthritis Rheum.* 1999;42:2123-2131.

15. Hennerbichler A, Moutos FT, Hennerbichler D, Weinberg JB, Guilak F. Interleukin-1 and tumor necrosis factor  $\alpha$  inhibit repair of the porcine meniscus in vitro. *Osteoarthritis Cartilage*. 2007;15:1053-1060.
16. Hill AM, Hoerning EJ, Brook K, et al. Collagenous microstructure of the glenoid labrum and biceps anchor. *J Anat*. 2008;212:853-862.
17. Hintermann B, Gächter A. Arthroscopic findings after shoulder dislocation. *Am J Sports Med*. 1995;23:545-551.
18. Kirkley A, Griffin S, Richards C, Miniaci A, Mohtadi N. Prospective randomized clinical trial comparing the effectiveness of immediate arthroscopic stabilization versus immobilization and rehabilitation in first traumatic anterior dislocations of the shoulder. *Arthroscopy*. 1999;15:507-514.
19. Kohn D. The clinical relevance of glenoid labrum lesions. *Arthroscopy*. 1987;3:223-230.
20. Liu SH, Panossian V, al-Shaikh R, et al. Morphology and matrix composition during early tendon to bone healing. *Clin Orthop Relat Res*. 1997;339:253-260.
21. Maini RN, Elliott M, Brennan FM, Williams RO, Feldmann M. TNF blockade in rheumatoid arthritis: implications for therapy and pathogenesis. *APMIS*. 1997;105:257-263.
22. Merino R, Gañan Y, Macias D, Economides AN, Sampath KT, Hurler JM. Morphogenesis of digits in the avian limb is controlled by FGFs, TGF $\beta$ s, and noggin through BMP signaling. *Dev Biol*. 1998;200:35-45.
23. Molloy T, Wang Y, Murrell G. The roles of growth factors in tendon and ligament healing. *Sports Med*. 2003;33:381-394.
24. Nho SJ, Frank RM, Van Thiel GS, et al. A biomechanical analysis of shoulder stabilization: posteroinferior glenohumeral capsular plication. *Am J Sports Med*. 2010;38:1413-1419.
25. Norlin R. Intraarticular pathology in acute, first-time anterior shoulder dislocation: an arthroscopic study. *Arthroscopy*. 1993;9:546-549.
26. Ogilvie-Harris DJ, Wiley AM. Arthroscopic surgery of the shoulder. A general appraisal. *J Bone Joint Surg Br*. 1986;68:201-207.
27. Prodromos CC, Ferry JA, Schiller AL, Zarins B. Histological studies of the glenoid labrum from fetal life to old age. *J Bone Joint Surg Am*. 1990;72:1344-1348.
28. Ribbans WJ, Mitchell R, Taylor GJ. Computerised arthrotomography of primary anterior dislocation of the shoulder. *J Bone Joint Surg Br*. 1990;72:181-185.
29. Roberts AB, Sporn MB. Transforming growth factor- $\beta$ . In: Clark RAF, ed. *The Molecular and Cellular Biology of Wound Repair*. 2nd ed. New York, NY: Plenum Press; 1995:265-275.
30. Schneeberger AG, Nyffeler RW, Gerber C. Structural changes of the rotator cuff caused by experimental subacromial impingement in the rat. *J Shoulder Elbow Surg*. 1998;7:375-380.
31. Shah M, Foreman DM, Ferguson MW. Neutralisation of TGF- $\beta$  1 and TGF- $\beta$  2 or exogenous addition of TGF- $\beta$  3 to cutaneous rat wounds reduces scarring. *J Cell Sci*. 1995;108:985-1002.
32. Shah M, Foreman DM, Ferguson MW. Neutralising antibody to TGF- $\beta$  1,2 reduces cutaneous scarring in adult rodents. *J Cell Sci*. 1994;107:1137-1157.
33. Soslowsky LJ, Carpenter JE, DeBano CM, Banerji I, Moalli MR. Development and use of an animal model for investigations on rotator cuff disease. *J Shoulder Elbow Surg*. 1996;5:383-392.
34. Stevens MM, Marini RP, Martin I, Langer R, Prasad Shastri V. FGF-2 enhances TGF- $\beta$ 1-induced periosteal chondrogenesis. *J Orthop Res*. 2004;22:1114-1119.
35. Taylor DC, Arciero RA. Pathologic changes associated with shoulder dislocations. Arthroscopic and physical examination findings in first-time, traumatic anterior dislocations. *Am J Sports Med*. 1997;25:306-311.
36. Thomas SC, Matsen FA 3rd. An approach to the repair of avulsion of the glenohumeral ligaments in the management of traumatic anterior glenohumeral instability. *J Bone Joint Surg Am*. 1989;71:506-513.
37. van den Berg WB, Joosten LA, van de Loo FA. TNF  $\alpha$  and IL-1  $\beta$  are separate targets in chronic arthritis. *Clin Exp Rheumatol*. 1999;17(6 suppl 18):S105-S114.
38. Wilusz RE, Weinberg JB, Guilak F, McNulty AL. Inhibition of integrative repair of the meniscus following acute exposure to interleukin-1 in vitro. *J Orthop Res*. 2008;26:504-512.
39. Wolf RS, Zheng N, Iero J, Weichel D. The effects of thermal capsulorrhaphy and rotator interval closure on multidirectional laxity in the glenohumeral joint: a cadaveric biomechanical study. *Arthroscopy*. 2004;20:1044-1049.