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In vivo kinetic evaluation of an adhesive capsulitis model in rats

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Background and Hypothesis: We hypothesized that extra-articular, internal fixation of the shoulder in rats would result in a subsequent decrease in rotational range of motion (ROM) and an increase in joint stiffness. We further hypothesized that residual kinematic changes would still be present at 8 weeks after immobilization. Extra-articular, internal fixation of the shoulder has been used to induce adhesive capsulitis in rats; however, the effects on in vivo kinematics have not been assessed.
Methods: Baseline measurements of rotational torque and ROM were acquired (n = 10 rats), and the left forelimb of each animal was immobilized with sutures passed between the scapula and the humeral shaft. After 8 weeks, the sutures were removed, and changes in kinematics and kinetics were longitudinally quantified in the follow-up period. Changes in stiffness, defined as the area under the angle-torque curve, were also quantified.
Results: Immediately after suture removal, there was a 63% decrease in total ROM compared with baseline (51° ± 10° vs. 136° ± 0°; $P < .001$). Similarly, total torque was found to increase 13.4 N.mm compared with baseline (22.6 ± 5.9 N.mm vs. 9.2 ± 2.6 N.mm; $P = .002$). Residual total ROM restrictions and an increased torque in internal rotation were still evident at 8 weeks of follow-up (113° ± 8° vs. 137° ± 0°, $P < .001$ and 3.5 ± 0.4 N.mm vs. 2.7 ± 0.7 N.mm, $P = .036$). Stiffness also increased after suture removal and at 8 weeks of follow-up compared with baseline.
Conclusion: This animal model of adhesive capsulitis rendered lasting effects on in vivo kinematics of the shoulder.
Level of evidence: Basic Science Study, In Vivo Animal Study.

Keywords: Adhesive capsulitis; frozen shoulder; contracture; animal model; biomechanics; rat

Adhesive capsulitis is characterized by pain, stiffness, and a prolonged loss of range of motion (ROM) in the glenohumeral joint and affects 2% to 5% of the U.S. population.[8,13,15] This disease is pathologically described by a fibrotic capsule that demonstrates a chronic inflammatory

This study was approved by the Beth Israel Deaconess Medical Center's Institutional Animal Care and Use Committee: Protocol No. 053-2013.

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infiltrate, absence of synovial lining, and subsynovial fibrosis.[14,16,21] Most accounts describe a highly vascularized synovium with abundant granulation tissue that may intrude on the bursa.[2,3] Immunohistochemistry has confirmed the presence of T cells, B cells, synovial cells, fibroblasts, and transforming myofibroblasts along with type I and type III collagen in biopsy samples collected from patients undergoing surgical capsular release.[4,16] However, the exact etiology and natural history of the disease remain uncertain.

Adhesive capsulitis is often described as a self-limited disease, but the course of recovery is arduous and long.[5] Although some patients achieve spontaneous resolution, a significant number fail to recover full ROM after treatment, and up to 60% have residual restrictions of motion years after the onset of symptoms.[18] It has been reported that mild to moderate symptoms can persist after 4.4 years following symptom onset of primary adhesive capsulitis. For those experiencing severe disease, such functional impairment may interfere with daily activities.[6]

Although it is believed that adhesive capsulitis can be treated with physical therapy, the best mode of treatment has been the subject of extreme interest. To evaluate the efficacy of new therapeutic options, it is necessary to develop an accurate animal model of adhesive capsulitis that will achieve the 2 hallmark characteristics of the disease: capsular contracture and a prolonged reduction in ROM. Previous studies have demonstrated that synovial shortening and deposition of type III collagen in the subsynovial area, both typical pathologic findings in adhesive capsulitis, appear in rats after 8 weeks of extra-articular, internal fixation of the glenohumeral joint.[10,12] These studies reportedly achieved a reduction in ROM with this animal model of adhesive capsulitis, as demonstrated by ex vivo measurements of shoulder kinematics. However, they failed to report whether these changes are transient or permanent.

No studies to date have evaluated whether this animal model of adhesive capsulitis affects in vivo shoulder kinetics (i.e., stiffness). Therefore, the aim of this study was to investigate the effects of extra-articular, internal fixation of the glenohumeral joint on shoulder kinetics and kinematics in an in vivo animal model of adhesive capsulitis. To that end, we hypothesized that rotational ROM would decrease and joint stiffness would increase after an 8-week period of immobilization. We further hypothesized that a spontaneous complete recovery would not occur and therefore residual kinematic and kinetic changes would still be present after 8 weeks of follow-up.

Materials and methods

Study design

Following approval by the Institutional Animal Care and Use Committee, a total of 10 Sprague-Dawley rats (250-300 g; Charles River Laboratories, Wilmington, MA, USA) were used in this study. For each animal, torque was measured per degree on the intact left shoulder as a function of rotation angle between 80° of internal rotation (negative values by convention) and 60° of external rotation (positive values by convention) before any surgical intervention (baseline). Rotation was confined within boundaries that were observed to elicit minimal scapular recruitment, as confirmed by fluoroscopy. Torque values at 80° of external rotation (τ_{OUT}) and 60° of internal rotation (τ_{INT}) were recorded for each animal.

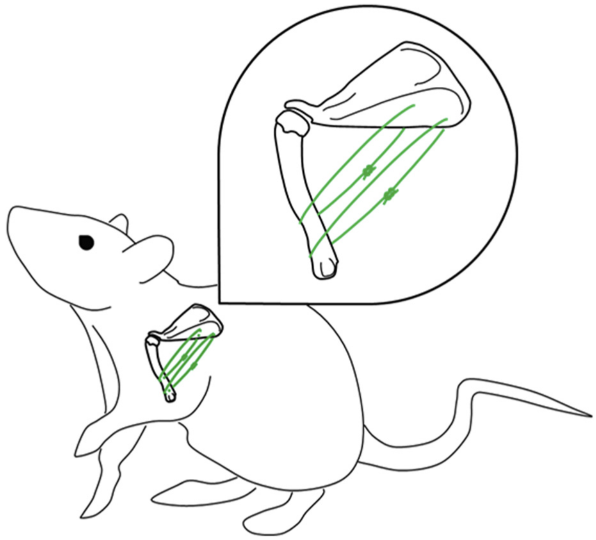

Figure 1 Schematic representation of the model of extra-articular, internal fixation of the glenohumeral joint. Braided polyester sutures were used to firmly tie the scapular edge to the distal third of the humerus.

The left forelimb of each animal was immobilized with a version of the procedure developed by Kanno et al.[10] Briefly, anesthesia was induced with 5% isoflurane inhalation and then maintained with 2% isoflurane. A longitudinal skin incision was made perpendicular to the scapular spine. Two No. 2-0 braided polyester sutures (Ethibond Excel; Ethicon, San Lorenzo, PR, USA) were passed between the medial border of the scapula and the humeral shaft and tightened to immobilize the shoulder joint (Fig. 1). Muscle structures were not manipulated during surgery, and the animals were allowed normal activity in their cages immediately after the procedure.

After 8 weeks of immobilization, the restraining sutures were removed, and the 10 animals were divided into 2 groups to evaluate changes in ROM (ROM group, n = 5) and joint stiffness (stiffness group, n = 5). In the ROM group, changes in kinematics were longitudinally quantified in the follow-up period by measuring the ROM achieved with the τ_{OUT} and τ_{INT} measured at baseline. This was conducted to evaluate whether immobilization mediated a significant reduction in ROM. In the stiffness group, joint kinetics were examined by measuring the differences in τ_{OUT} and τ_{INT} needed to achieve the original 80° of internal rotation and 60° of external rotation, respectively. Measurements for both groups were taken immediately after suture removal (day 0 of follow-up) and at regular intervals thereafter (twice a week until $<$10% change was observed in 3 consecutive time

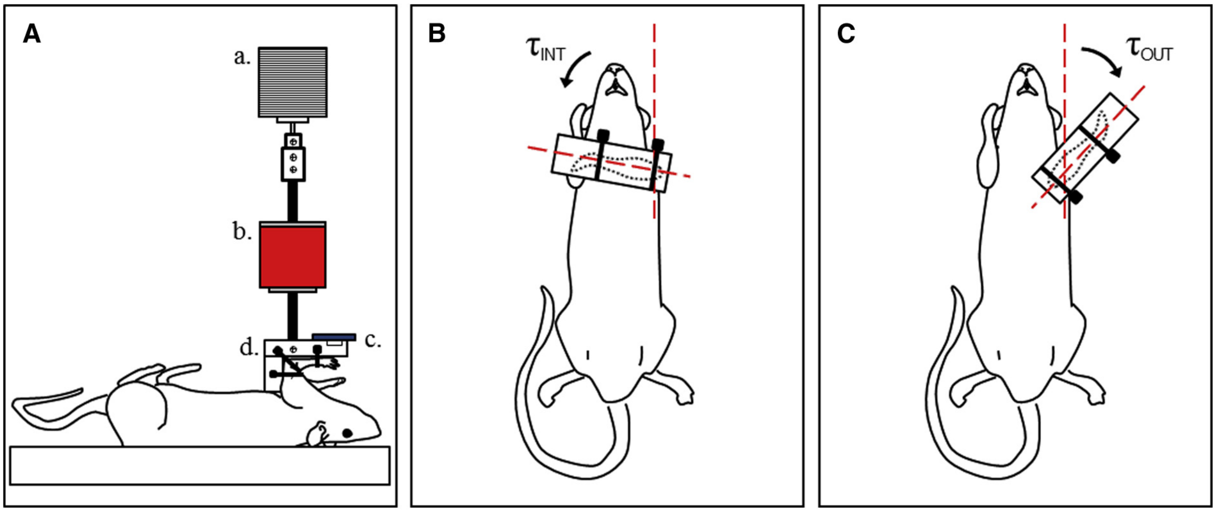

Figure 2 (**A**) Schematic representation of the measuring device. The animal's forelimb was attached to the arm clamp (*d*), while the stepper motor (*a*) provided the driving force for the ROM measurement. The sensor assembly consists of an orientation sensor (*c*) and a reaction torque sensor (*b*). Rotation of the sensor assembly resulted in direct internal (**B**) or external (**C**) humeral rotation within the glenohumeral joint.

points, at which point measurement frequency was reduced to once a week).

The baseline measurements for each group were used as internal controls to reduce the total number of animals necessary to conduct the study. The use of internal controls also increased internal validity and statistical power as there is a high interspecimen variation, of both ROM and measured torques, even when the contralateral shoulder of the same animal is used. Finally, a pilot study demonstrated that intraspecimen measurements are highly reproducible and remain stable during an 8-week period.

Range of motion and joint stiffness measurements

ROM and torque measurements were performed, under general anesthesia as described before, with a modified version of the device reported by Sarver et al.[17] The device consisted of a sensor assembly, a rotating axle, and an arm clamp. The sensor assembly contained an orientation sensor with 3° of freedom (3DM-GX3-15; MicroStrain Inc., Williston, VT, USA) as well as a reaction torque sensor (TFF400; Futek Inc., Irvine, CA, USA), all secured to the axle such that the sensing axis was collinear with the center of rotation. The animal's forelimb was secured to the arm clamp at 3 points (wrist, elbow, and arm), ensuring that the sensing axis was aligned with the long axis of the animal's humerus (Fig. 2, *A*). As a result, rotation of the sensor assembly resulted in direct external/internal humeral rotation within the glenohumeral joint (Fig. 2, *B* and *C*).

To reproducibly capture ROM and torque, passive limb rotation was performed by a stepper motor controlled with a microcontroller (UNO R3; Arduino, Torino, Italy). The system used inputs from the reaction torque sensor or the orientation sensor to start and to end the dynamic measurement of ROM and torque. In the ROM group, preset programmable torque values, specific for each animal and measured at baseline (τ_{OUT} and τ_{INT}), were used as input variables to detect changes in rotation ROM with 0.2° resolution. In the stiffness group, preset programmable rotation angles (60° external rotation, 80° internal rotation) were used as input to measure changes in torque at a resolution of 0.01 N/mm. The microcontroller was fed directions from a computer using MATLAB 7.13.0.564 (MathWorks Inc., Natick, MA, USA).

Data and statistical analysis

While either ROM or torque was recorded, each trial was repeated 3 times in each animal to ensure consistency. Results for ROM and stiffness are shown as averages with standard error. Stiffness values were normalized with respect to baseline measurements for ease of visualization and processing.

In the ROM group, mean ROM values were compared at 3 different time points (baseline, immediately after suture removal, and at 8 weeks of follow-up) by repeated-measures analysis of variance. A value of $P < .05$ was considered statistically significant.

In the stiffness group, 2 different metrics were used for comparison: (1) the difference in torque required to achieve full ROM and (2) the corresponding stiffness calculations. The torque was used as a direct measure for effort to discover the change in force required for a full ROM after immobilization. Stiffness can be considered the relation between force and the amount of deformation[17,22] and was defined with units of torque per angle, N.mm/degree, or as the slope of the rotation angle-torque curve. Because of the lack of strong linear fit, this slope was best approximated by a cubic polynomial. Because stiffness was calculated as a third-order polynomial, the area under the curve (AUC) was compared between 3 different time points (baseline, immediately after suture removal, and at 8 weeks of follow-up) by the Wald slope test. A value of $P < .05$ was considered statistically significant.

Results

Range of motion group

The temporal behavior of ROM in the follow-up period is shown in Figure 3. After extra-articular internal fixation of the glenohumeral joint, the first measurement (day 0 of follow-up; Fig. 3, point B) showed a 63% decrease in total ROM from baseline (Fig. 3, point A) (136° ± 0° vs. 51° ± 10° for baseline and day 0 of follow-up, respectively; $P < .001$). Gradual improvement was observed until week 5, when progression plateaued at 19% restriction (136° ± 0° vs. 108° ± 5° for baseline and 5 weeks of follow-up, respectively; $P < .001$). The residual restriction was still evident at 8 weeks of follow-up (18% restriction of total ROM, 136° ± 0° vs. 113° ± 8° for baseline and 8 weeks of follow-up, respectively; $P < .001$).

Stiffness group

Immediately after fixation removal, torque was found to increase 13.4 N.mm compared with baseline (Fig. 4, point B; 9.2 ± 2.6 N.mm vs. 22.6 ± 5.9 N.mm for baseline and day 0 of follow-up, respectively; $P < .001$). This includes an increase in torque of 8.9 N.mm externally (6.5 ± 1.4 N.mm vs. 15.4 ± 7.08 N.mm; $P = .002$) and 4.4 N.mm internally (2.7 ± 0.7 N.mm vs. 7.1 ± 1.3 N.mm; $P < .001$) (Fig. 5, point B), resulting in a 138.8% and a 159.6% increase in torque, respectively, totaling at 149.2% more torque overall (Fig. 6). At 8 weeks of follow-up, the total measured torque was 1.4 ± 0.2 N.mm higher than initial conditions (9.2 ± 1.8 N.mm vs. 10.6 ± 0.4 N.mm for baseline and 8 weeks of follow-up, respectively; $P = .115$), resulting from an increase of external torque of 0.6 ± 0.1 N.mm (6.5 ± 1.4 N.mm vs. 7.1 ± 0.3 N.mm for baseline and 8 weeks of follow-up, respectively; $P = .369$) and an increase of internal torque of 0.7 ± 0.2 N.mm (2.7 ± 0.7 N.mm vs. 3.5 ± 0.4 N.mm for baseline and 8 weeks of follow-up, respectively; $P = .036$) compared with baseline (Fig. 4). This resulted in a final increase in torque of 17.9%, with increases of 10.0% externally and 25.7% internally (Fig. 6). Unlike the kinematic measurements, where the angular improvement plateaued at around 5 weeks, the kinetic data showed an earlier plateau at the beginning of 3 weeks.

The loading curves for external and internal rotations were fitted with cubic polynomials as described in Table I, with all fits being statistically significant from each other, the exception being between baseline and follow-up measurements for external rotation. Statistics for the comparison between slopes can be found in Table II. The AUCs of external and internal rotation after suture removal, 744.7 and 200.6, respectively, were found to be much higher than the AUCs of baseline, which were 318.4 and 93.6, respectively (Table I). Comparing baseline AUC with the 8-week follow-up AUC also shows a similar increase with 342.1 externally and 113.6 internally (Fig. 7). The differences in slopes described by the AUC are apparent and can be seen in Figure 8.

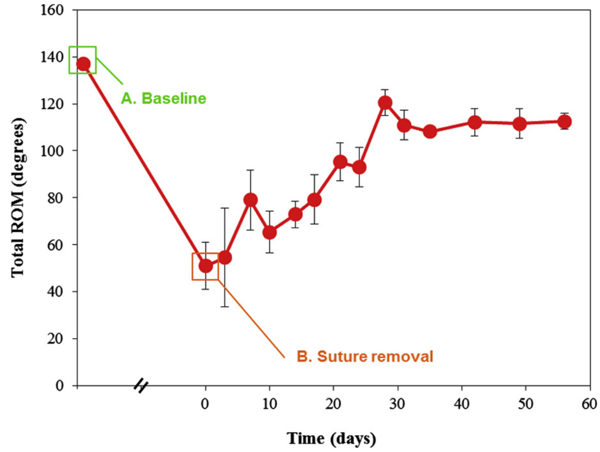

Figure 3 Change in range of motion (*ROM*) over time. Results are presented as means with standard errors used as a measure of dispersion. Point A corresponds to the measurement taken at baseline, whereas point B corresponds to the measurement immediately after suture removal. The final point on this plot corresponds to the final follow-up measurement.

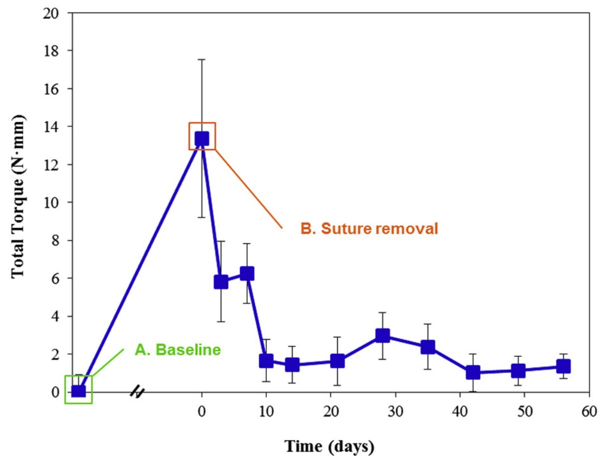

Figure 4 Change in overall torque over time. Results are presented as means with standard errors used as a measure of dispersion. Point A is the measurement taken at baseline, and point B is the measurement immediately after suture removal. The final point on this plot corresponds to the final follow-up measurement.

Discussion

An animal model of adhesive capsulitis must achieve the 2 hallmark characteristics of the disease: capsular contracture and a prolonged reduction in ROM. Kanno

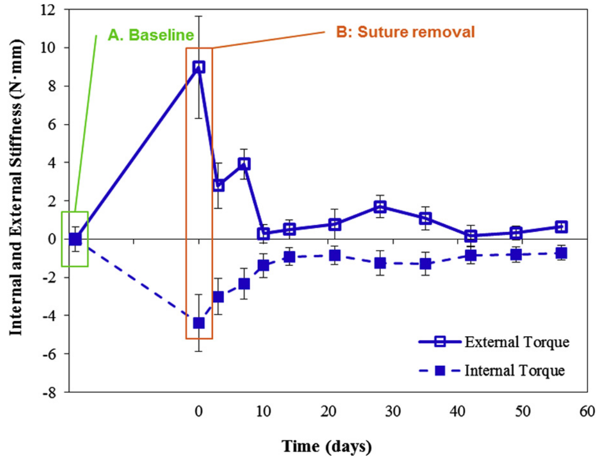

Figure 5 Change in torque of internal and external rotation over time. Results are presented as means with standard errors used as a measure of dispersion. Point A is the measurement taken at baseline, and points B are the measurements immediately after suture removal. The final points on this plot correspond to the final follow-up measurement.

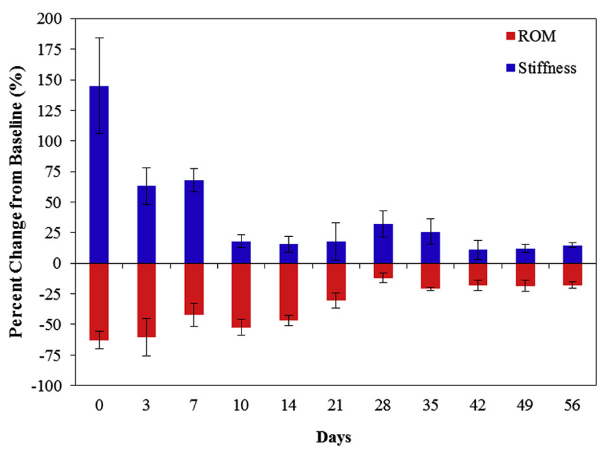

Figure 6 An overall comparison between range of motion (*ROM*) and torque changes as percentages. Results are presented as means with standard errors used as a measure of dispersion. ROM is represented as a negative change, whereas stiffness is shown as an increased change.

et al[10] developed a rat model of adhesive capsulitis by immobilizing the glenohumeral joint with extra-articular internal fixation. This model induced morphologic changes consistent with adhesive capsulitis and a reduction in shoulder ROM in abduction and total rotation. However, these kinematic changes were not evaluated in vivo but instead immediately after the removal of fixation implants. As a result, they were not able to meaningfully comment on the temporal behavior of the mechanical effect.

In concordance with our hypothesis, extra-articular internal fixation of the glenohumeral joint induced a reduction in total ROM for at least 8 weeks. During our kinematic assessments, τ_{OUT} and τ_{INT} measured at baseline were used for each animal to quantify the differences in ROM at different time points. Kanno et al,[10] on the other hand, used a standardized torque (3.96×10^{-3} N.m) in all animals to prompt ex vivo assessments on ROM. Despite these methodologic differences, our findings were in agreement with the restriction pattern observed by Kanno et al immediately after the removal of immobilization (62.85% vs. 60.03%). Of note, the magnitude in ROM immediately after immobilization differed, presumably because of the difference in torques applied (51° ± 10° vs. 17° ± 5°).

Extra-articular internal fixation of the glenohumeral joint also resulted in increased shoulder joint stiffness. Because of the lack of documented analysis on the overall kinetics of this model, these results cannot be longitudinally compared. Nevertheless, Sarver et al[17] described the resultant stiffness of the shoulder joint after nonsurgical external fixation of the shoulder. More specifically, this study demonstrated that the increase in joint stiffness (defined as the slope of the linear portion of the angle-torque curve) caused by immobilizing an injured and repaired shoulder was transient for external rotation at 4 weeks yet resolved by 8 weeks of follow-up. In contrast, our study demonstrated that in the loading section of the curve, torque and angle do not demonstrate a linear relationship but instead correlate more strongly to a polynomial fit. Furthermore, statistically significant differences in kinetics with respect to baseline were observed only during internal rotation, where a sustained stiffness increase of 25.7% was observed after 8 weeks of immobilization.

The kinetic and kinematic changes found were not transitory. At 8 weeks of follow-up, both the reduction in ROM and the increase in joint stiffness were significant. Whereas no studies have evaluated the natural progression and temporal behavior of this model of adhesive capsulitis, it has been theorized that joint residual changes present after 8 weeks into the post-immobilization period are likely to be permanent.[19] This finding is of particular importance as a prolonged period of reduced ROM and increased stiffness without spontaneous recovery will allow the comprehensive evaluation of current and potential therapeutic interventions for adhesive capsulitis.

Although fibrosis, muscle atrophy, and other post-surgical changes are likely to contribute to joint stiffness and limitations in ROM, such contributions would likely be negligible after 8 weeks of caged activity. In their kinematic analysis of the immediate postimmobilization period, Kanno et al reported that myotomy of the shoulder girdle did not have an effect on passive shoulder ROM. The authors concluded that the capsule, specifically the anterosuperior and anteroinferior portions, was the major

Table I Coefficients of fitted cubic stiffness equations and their corresponding areas under the curve

Motion Type	Third-order coefficient	Second-order coefficient	First-order coefficient	Constant	R^2 value	AUC	95% Confidence interval
External rotation							
Baseline	0.05	0.24	1.72	3.24	0.99	318.3	315.1-321.5
After suture removal	0.05	0.74	4.38	7.13	0.99	744.7	739.1-750.3
Follow-up	0.11	0.36	1.71	3.37	0.99	342.1	337.8-346.3
Internal rotation							
Baseline	0.01	−0.06	0.69	−1.81	0.99	93.5	91.3-95.7
After suture removal	0.09	−0.26	1.83	−4.20	0.99	200.5	197.5-203.6
Follow-up	0.07	−0.17	0.92	−2.26	0.99	113.6	110.5-116.7

AUC, area under the curve.

Table II Comparison of area under the curve for angle vs. torque as fitted by cubic polynomial regression*

Motion Type	Wald test (slope test)	*P* value
External rotation		
Baseline vs. after suture removal	8.56	.003†
Baseline vs. follow-up	0.02	.894
After suture removal vs. follow-up	29.73	<.001†
Internal rotation		
Baseline vs. after suture removal	68.04	<.001†
Baseline vs. follow-up	7.85	.005†
After suture removal vs. follow-up	120.87	<.001†

* Area under the curve was compared by the slope test using generalized estimating equations.
† Statistically significant.

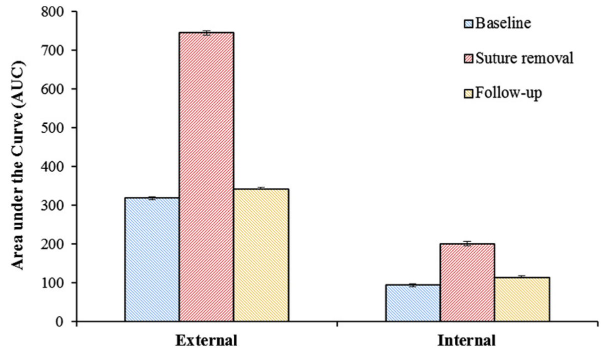

Figure 7 Area under the curve for both external and internal cubic stiffness equations. *Error bars* correspond to the 95% confidence intervals of the polynomial fit.

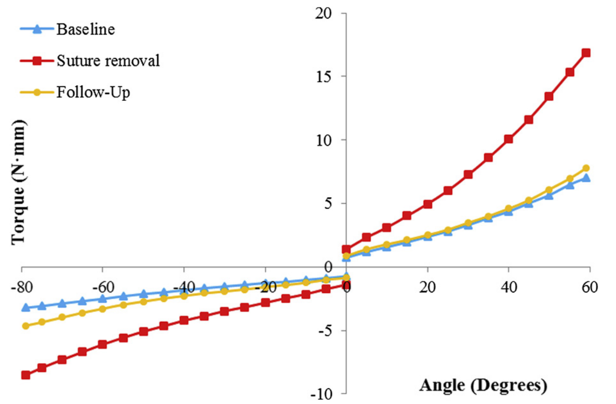

Figure 8 Loading curves of external and internal rotation per condition. Negative angles denote internal rotation and positive angles denote external rotation.

culprit for the variations in kinematics.[10] Similarly, Trudel et al have investigated the relative contributions of muscular and articular structures to joint contracture and reported that muscle atrophy significantly contributed to ROM reduction only in early-onset contractures (immobilization periods of 1 or 2 weeks), whereas in late-onset contractures (≥4 weeks of fixation), the articular structures were primarily responsible for the irreversible kinematic changes—after an immobilization period of 8 weeks, the articular structures were responsible for 90% of the reduction in ROM immediately after the removal of the fixation devices and for 100% of the reduction in ROM at any time point thereafter (8, 16, or 32 weeks).[19,20] This evidence suggests that the kinematic changes induced in this model are likely due to changes in the synovial capsule.

Limitations

As with any study, several limitations must be considered in interpreting these results. First, caution must be exerted when results are translated from animal models, especially in this case as the shoulder in quadruped animals is a weight-bearing joint. In addition, it is not possible to completely isolate glenohumeral motion from scapulothoracic motion during in vivo kinematic measurements without rigidly fixing the scapula. This issue was addressed by assessing scapulothoracic motion during humeral rotation with fluoroscopy and consequently identifying boundary conditions for kinematic and kinetic evaluations, where scapular recruitment was initiated at approximately 60° of external rotation and 80° of internal rotation. We acknowledge that the selection of these boundaries followed a semiquantitative process and that further studies are warranted to objectively quantify the relative contributions of glenohumeral and scapulothoracic motion to shoulder rotation.

Unlike previous studies of joint contracture kinematics, because of the in vivo nature of our experiments, we did not quantify the differential contributions of muscular and articular elements. Instead of using a fixation plate as described in the original model by Kanno et al,[10] we achieved extra-articular internal fixation with use of extraperiosteal sutures between the scapula and the distal third of the humerus to avoid undue damage to muscular structures. This method cannot provide complete rigid fixation, and some degree of adduction may be present if the sutures are loose. However, by firmly tightening the sutures, the forelimb was fastened to the lateral chest wall in a maximally adducted position. Finally, only passive rotational mechanic changes were quantified in this study, as the behavior of the joint in other rotational and translational degrees of freedom would require a more complicated device. Nevertheless, the current device obtains passive mechanical data in an orientation and about an axis that is used frequently to assess shoulder function.

The pathogenesis of idiopathic adhesive capsulitis remains poorly understood. Some authors have suggested that there might be an inflammatory trigger that leads to the dense fibrosis of the joint capsule.[7] However, regardless of the etiology, the cause of painful restriction of movement is the contracture of the capsule, which reduces the available intra-articular volume, thus limiting glenohumeral movement.[1,3,9,11] This animal model cannot replicate the initial inflammatory insult of idiopathic inflammatory adhesive capsulitis. However, it accurately mimics the pathologic changes to the joint capsule[10,12] and to glenohumeral kinetics that are characteristic of the disease.

Conclusion

An animal model simulating adhesive capsulitis by glenohumeral joint immobilization using extra-articular, internal fixation rendered lasting effects (for at least 8 weeks) on in vivo kinematics of the shoulder. More specifically, operative immobilization yielded a lasting reduction in total ROM and also an increase in joint stiffness, which are likely the result of changes in the synovial capsule.

Disclaimer

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