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## Original Article

# Influence of corticoids on healing of the rotator cuff of rats – biomechanical study<sup>☆,☆☆</sup>

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## ABSTRACT

**Objective:** to compare healing strength of the infraspinatus tendon of rats with corticoid inoculation, regarding maximum tension, maximum force and rupture force, after injury and experimental repair.

**Methods:** a total of 60 Wistar rats were subjected to tenotomy of the infraspinatus tendon, which was then sutured. Before the surgery, they were divided into a control group (C) inoculated with serum and a study group (S) inoculated with corticoids over the tendon. After repair, the rats were sacrificed in groups of 10 individuals in the control group and 10 in the study group at the times of one week (C1 and S1), three weeks (C3 and S3) and five weeks (C5 and S5). The rats were dissected, separating out the infraspinatus tendon with the humerus. The study specimens were subjected to a traction test, with evaluation of the maximum tension (kgf/cm<sup>2</sup>), maximum force (kgf) and rupture force (kgf), comparing the study group with the respective control groups.

**Results:** among the rats sacrificed one week after the procedure, we observed greater maximum tension in group C1 than in group S1. The variables of maximum force (kgf) and rupture force did not differ statistically between the groups investigated. In the same way, among the rats sacrificed three weeks after the procedure, group C3 only showed greater maximum tension than group S3 ( $p = 0.007$ ), and the other variables did not present differences. Among the rats sacrificed five weeks after the procedure (C5 and S5), none of the parameters studied presented statistical differences.

**Conclusion:** we concluded that corticoid diminished the resistance to maximum tension in the groups sacrificed one and three weeks after the procedure, in comparison with the respective control groups. The other parameters did not show differences between the study and control groups.

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## Influência do corticoide na cicatrização do manguito rotador de ratos – Estudo biomecânico

### R E S U M O

#### Palavras-chave:

Manguito rotador  
Corticoides  
Biomecânica  
Tendões

**Objetivo:** comparar a resistência da cicatrização, com relação a tensão máxima, força máxima e força de ruptura, do tendão infraespinhal de ratos submetidos a inoculação de corticoides após a lesão e a reparos experimentais.

**Métodos:** foram submetidos 60 ratos Wistar a tenotomia do tendão infraespinhal e suturados. Previamente à cirurgia foram divididos em grupo controle (C), inoculados com soro, e grupo de estudo (E), inoculados com corticoides sobre o tendão. Após o reparo os ratos foram sacrificados em grupos de 10 indivíduos do grupo controle e 10 do grupo de estudo em intervalos de uma semana (C1 e E1), três semanas (C3 e E3) e cinco semanas (C5 e E5). Os ratos foram dissecados com a separação do tendão infraespinhal do úmero. As peças de estudo foram submetidas a teste de tração e avaliadas – tensão máxima (kgf/cm<sup>2</sup>), força máxima (kgf) e força de ruptura (kgf) – e comparando os grupos de estudo com os grupos controle.

**Resultados:** dentre os ratos sacrificados com uma semana observamos maior tensão máxima do grupo C1 em comparação com o grupo E1. As variáveis força máxima (kgf) e força de ruptura (kgf) não diferiram estatisticamente entre os grupos pesquisados. Da mesma forma, nos ratos sacrificados com três semanas o grupo C3 mostrou apenas resistência maior na tensão máxima em comparação com o grupo E3 ( $p=0.007$ ). As demais variáveis não apresentaram diferenças. Nos ratos sacrificados com cinco semanas (C5 e E5), nenhum dos parâmetros estudados apresentou diferenças estatísticas.

**Conclusão:** a inoculação com corticoide sobre o manguito rotador levou a diminuição da resistência a tensão máxima da cicatriz pós reparo cirúrgico experimental em uma e três semanas em comparação com os respectivos grupos controle. Os demais parâmetros não tiveram diferença entre os grupos de estudo e os grupos controle.

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## Introduction

Rotator cuff disease is frequently seen in medical practice. It comprises a spectrum of conditions ranging from an inflammatory process in the tendon to complete rupture of the rotator cuff.<sup>1,2</sup>

Subacromial infiltration of corticoid is a treatment option in cuff injuries in patients with low functional demands and also as a therapeutic resource for temporary pain relief in active patients.<sup>1,3-5</sup>

Gray and Gottlieb<sup>6</sup> studied the prognostic factors for rotator cuff repair and showed that use of three or more preoperative infiltrations of corticoids was related to a higher repair failure rate. Likewise, Watson<sup>7</sup> demonstrated that the more frequent the use of corticosteroids was, the worse the result was, particularly from the fourth infiltration onwards, and recommended that surgery should be performed before the fourth infiltration. In another evaluation, Björkenheim et al.<sup>8</sup> showed that, among the cases of failure of surgical repair of rotator cuff injuries, 63% of the patients had received three or more corticoid injections. The remaining 37% had had two injections or less.

Furthermore, experimental studies on animals have shown histological changes and diminished resistance in tendons that were subjected to corticoid exposure.<sup>9-15</sup> There is also evidence that corticoid use may alter the resistance of the tendon repair.<sup>16,17</sup> Studies that have assessed the influence of

corticosteroids on the rotator cuff have used undamaged tendons from rats or partially torn tendons.<sup>12-15</sup>

The present study was justified by the need to obtain objective data that might determine whether corticoid use might compromise the healing of surgical repairs to the rotator cuff.

The objective of this study was to evaluate the resistance of healed infraspinatus tendons from rats that were exposed to corticosteroids at different times (one, three and five weeks after suturing).

## Materials and methods

This project was submitted for approval by the ethics committee for animal research of Positive University.

Sixty female rats of the Wistar lineage of the species *Rattus norvegicus* were used. The mean weight of the rats was 300 g and their mean age was three months. The animals were kept in collective cages in the vivarium of Positive University, with free access to water and commercial feed. Throughout the experimental period, the environmental conditions of light, temperature and humidity in the rooms were controlled via a digital panel, which maintained a photoperiod of 12 h, temperature range from 18 to 22 °C and relative air humidity of 65%.

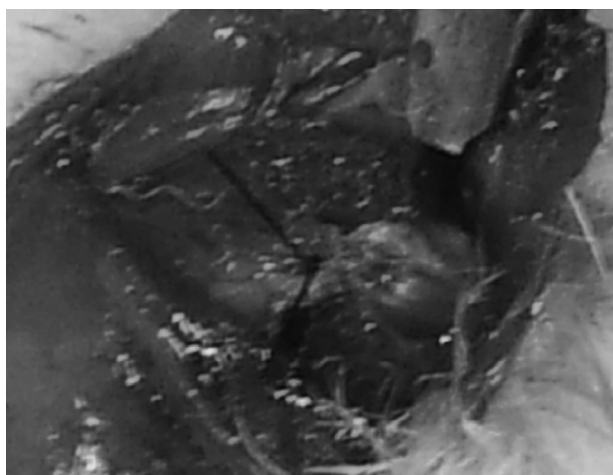
The rats were operated in groups of 20 animals per working day. It was standardized that only the right side would be operated. An incision of 1 cm was made in the lateral rim of the



**Fig. 1 – Sectioned infraspinatus tendon.**

acromion and the fibers of the deltoid were divided. After the infraspinatus tendon had been isolated, the medial portion of the body of the tendon was sectioned transversally using a no. 11 scalpel blade (Fig. 1). The tendon was then repaired using U-shaped stitches of vascular 5-0 mononylon thread (Fig. 2).

The rats that underwent the operation were randomized and distributed into two groups and were paired into 30 units: group C (control), in which, after tendon suturing, 0.5 mL of 0.9% physiological saline solution was inoculated into the sub-acromial space under direct viewing; and group D (study), in which, in the same manner, a single dose of 0.6 mg/kg of methylprednisolone (0.5 mL of prepared solution) was inoculated. Groups C and S were then subdivided into three subgroups according to the time when the animals were sacrificed for data collection: one week (groups S1 and C1), three



**Fig. 2 – Sutured infraspinatus tendon.**

**Table 1 – Subdivision of the control groups (C) and study groups (S) according to the time when the rats were sacrificed.**

Group	Inoculation	Time until sacrifice, in weeks
C1	Serum	1
S1	Corticoid	
C3	Serum	3
S3	Corticoid	
C5	Serum	5
S5	Corticoid	

weeks (groups S3 and C3), and five weeks (groups S5 and C5) (Table 1).

The corticoid preparation used was Depo-Medrol® (methylprednisolone acetate), in a solution of 80 mg/2 mL. Half an ampoule (1 mL) of the medication was diluted in 100 mL of physiological saline solution, and the solution volume became 0.4 mg/mL.

All the rats were sacrificed in a chamber containing CO<sub>2</sub>.

After the sacrifice, dissection was performed immediately, by means of wide surgical access on the operated shoulder. The clavicle was resected and the tendons and ligaments were sectioned, with separation of the shoulder from the rest of the animal's body. Each specimen was prepared with separation of the infraspinatus muscle from the remainder of the scapula. The test specimen consisted only of the infraspinatus (muscle and tendon gripped at their insertion into the rat's humerus).

Each dissected specimen was wrapped in gauze that had been soaked in 0.9% physiological saline solution and was isolated in a properly identified individual flask and placed in a freezer at minus 20 °C.<sup>18</sup>

The tendons were taken out of the freezer in groups of 20 specimens for defrosting over a 12-hour period at room temperature, before the biomechanical test.<sup>15</sup> After each specimen had been defrosted, its thickness was measured using a pachymeter, at its central point. The thinnest part of the transverse section was used for the measurement.<sup>19</sup> The area of the tendon was calculated to determine the maximum tension value, given in kgf/cm<sup>2</sup>.

The apparatus for the biomechanical tests was the Emic model DL 500 MF, with a load cell of 50N and axial traction force. The machine's software supplied the parameters of maximum tension (kgf/cm<sup>2</sup>), maximum force (kgf) and rupture force (kgf) (Fig. 3).

The tests were performed as described by Galatz et al.<sup>20</sup> Each defrosted specimen, composed of the humerus and the isolated infraspinatus tendon, was fixed one at a time in the machine, for traction. The fixation was done using the clasps of the machine: the humerus was fixed using the base clasp (fixed part) and the tendon was fixed with the clasp that was attached to the load cell. For the tendon to be adequately gripped by the clasp, ordinary sandpaper of medium roughness was used, glued onto both sides of the clasp using removable adhesive.

For the statistical analysis, the assumption of normal distribution of the data obtained was tested, i.e. maximum tension (kgf/cm<sup>2</sup>), maximum force (kgf) and rupture force (kgf). For this purpose, the Shapiro-Wilk statistical test was used, in



**Fig. 3 – Axial traction test apparatus with 50 N load cell.**

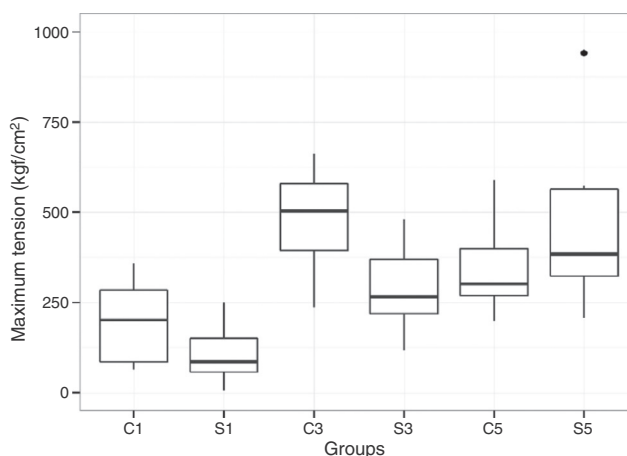
which the nullity hypothesis expresses that the data in question present normal distribution. The data were also used to configure a boxplot graph. Following this, the t statistical test for independent samples was performed, taking  $p < 0.05$ . The results obtained regarding maximum force (kgf), rupture force (kgf) and maximum tension (kgf/cm<sup>2</sup>), from both preparations, were compared.

**Results**

The sample studied comprised 57 infraspinatus tendons from rats. In three rats, it was not possible to obtain the tendons due to premature death: one in group C1, one in C3 and one in S1.

All the tendons broke at the healing site. The results from the biomechanical tests are presented in tables, subdivided between the respective groups (Tables 2–4). The distribution of the boxplot graph values is presented in Figs. 4–6.

In the one-week study groups, greater maximum tension ( $p = 0.03$ ) was observed in group C1 than in group S1. The variables of maximum force (kgf) and rupture force (kgf) did not differ statistically between the groups investigated. Likewise, in the three-week groups, group C3 showed greater resistance than group S3, only in relation to maximum tension ( $p = 0.007$ ). There were no differences between the groups for the other variables. In the groups C5 and S5, none of the parameters studied presented any statistical differences.



**Fig. 4 – Boxplot showing distribution of the maximum tension among the groups.**

**Table 2 – Results obtained in relation to maximum tension, maximum force and rupture force, with their respective means and standard deviations for the groups C1 and S1 (one week).**

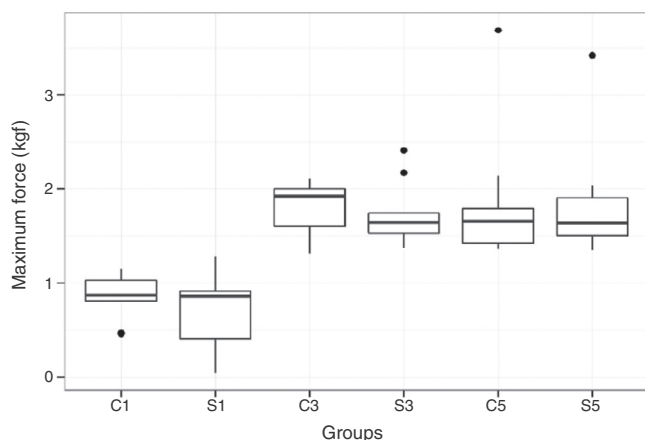
	Maximum tension (kgf/cm <sup>2</sup> )		Maximum force (kgf)		Rupture force (kgf)	
	C1	S1	C1	S1	C1	S1
Mean (SD)	208.17 (SD ± 113.84)	100.99 (SD ± 73.28)	0.85 (SD ± 0.25)	0.73 (SD ± 0.41)	0.95 (SD ± 0.15)	0.61 (SD ± 0.39)
Statistical difference	$p = 0.03$	$p > 0.05$	$p > 0.05$			

**Table 3 – Results obtained in relation to maximum tension, maximum force and rupture force, with their respective means and standard deviations for the groups C3 and S3 (three weeks).**

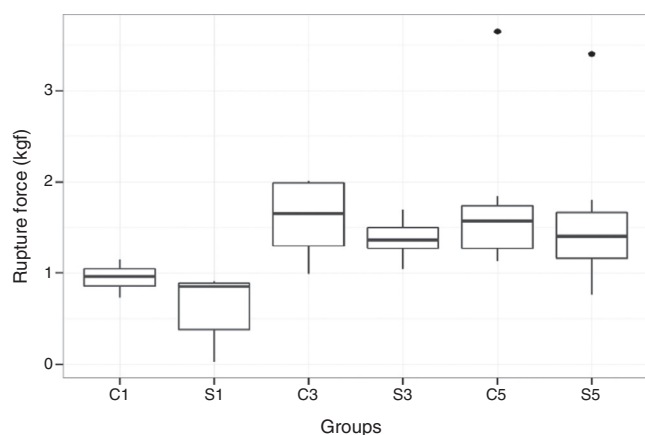
	Maximum tension (kgf/cm <sup>2</sup> )		Maximum force (kgf)		Rupture force (kgf)	
	C3	S3	C3	S3	C3	S3
Mean (SD)	476.26 (SD ± 157.85)	284.14 (SD ± 112.41)	1.78 (SD ± 0.32)	1.72 (SD ± 0.33)	1.63 (SD ± 0.39)	1.38 (SD ± 0.22)
Statistical difference	$p = 0.007$	$p > 0.05$	$p > 0.05$			

**Table 4 – Results obtained in relation to maximum tension, maximum force and rupture force, with their respective means and standard deviations for the groups C5 and S5 (five weeks).**

	Maximum tension (kgf/cm <sup>2</sup> )		Maximum force (kgf)		Rupture force (kgf)	
	C5	S5	C5	S5	C5	S5
Mean (SD)	340.26 (SD ± 118.78)	450.57 (SD ± 219.47)	1.83 (SD ± 0.7)	1.83 (SD ± 0.61)	1.71 (SD ± 0.77)	1.55 (SD ± 0.77)
Statistical difference	$p > 0.05$	$p > 0.05$	$p > 0.05$			



**Fig. 5 – Boxplot of the distribution of maximum force among the groups.**



**Fig. 6 – Boxplot of the distribution of rupture force among the groups.**

## Discussion

The rotator cuff of rats is used in experiments because of its similarities with the human shoulder, and even in relation to elevation and rotation movements in various planes. When rats run, the cuff passes under the acromion repeatedly, which is comparable to humans' activities with the upper limbs at elevations of more than 90°. <sup>21</sup> Moreover, the infraspinatus tendon in rats of the species *Rattus norvegicus* presents anatomical similarities with the human infraspinatus tendon and is longer than the supraspinatus tendon. <sup>15</sup> For our experiment, it was considered to be the ideal test body.

There are other ways of testing tendon injuries, but we chose the infraspinatus tendon of Wistar rats because we believe that the model described by Mikolyzk et al. <sup>15</sup> was very suitable for our study. The fundamental difference between our proposal and their study related to the extent of the experimental injury. They tested the influence of the corticoid on undamaged tendons and on the healing achieved following injury affecting approximately 50% of the width of the

tendon. We tested the action of corticoid on complete rupture that underwent surgical repair. The two studies had the same objective, i.e. to evaluate the action of corticoid on the infraspinatus tendon in rats, but in different situations.

Phelps et al. <sup>10</sup> evaluated the resistance of the patellar tendons of rabbits that were subjected to infiltration once a week for three weeks and then sacrificed the animals 4–54 days after the last infiltration. They did not demonstrate any significant differences between the group inoculated with serum and the group inoculated with methylprednisolone. In our model, we observed diminished resistance of the healed tissue, one and three weeks after corticoid was administered to the tendon. Phelps et al. <sup>10</sup> only used undamaged tendons and did not describe any standard time for sacrifice after the infiltrations. In our study, these variables were controlled.

Vogel, mentioned by Paavola et al., <sup>22</sup> reported that there was an increase in the tensile strength of tendons after corticoid was inoculated around the tendon. That result was divergent from ours, and from other studies in the literature, but because it is impossible to obtain the original study, little is known about the methodology used.

The influence of corticoids on tendon healing has already been studied by several authors. Wrenn et al. <sup>16</sup> studied extensor tendons in dogs' paws and demonstrated that, in tests conducted three weeks after surgical repair of an experimental injury, high doses of intramuscular hydrocortisone (10 mg/kg) had decreased the resistance of the healed tissue by 40%. Nevertheless, they stated that, despite the diminished rupture force, the resistance of the healed tissue was sufficient for normal functioning of the animal's limb. We obtained similar results in relation to diminished resistance of the healed tissue, one and three weeks after an experimental injury to the infraspinatus tendon in rats. Technical differences such as whether the injection was local or intramuscular seem not to have influenced the final result.

However, in an evaluation of sutured experimental injuries to tendons in dogs, in which hydrocortisone was inoculated around the tendon in a single dose, in a method similar to that of our study on the infraspinatus in rats using methylprednisolone, Gonzalez <sup>17</sup> did not demonstrate any statistical difference in the resistance of the healed tissue, three weeks after the surgical repair. This may have been related to the type of corticoid and the animal model used.

The structure of tendons may become modified through corticoid use. Akpınar et al. <sup>13</sup> demonstrated that after four subacromial infiltrations with betamethasone or methylprednisolone, the tendons became macroscopically softer, lighter and discolored in comparison with tendons that were infiltrated with saline solution or even with normal tendons. Regarding the microscopic appearance, the groups inoculated with corticoids demonstrated fragmentation of the collagen fibers. These authors concluded that corticoids could cause deleterious effects in the rotator cuff tendons and did not recommend their clinical use for multiple infiltrations. Tillander et al. <sup>12</sup> used repeated subacromial infiltrations of triamcinolone in rat tendons. They observed that up to three infiltrations of triamcinolone did not produce any macroscopic or microscopic alterations in relation to the control group. In evaluating the group of rats that received five infiltrations, they observed that 28% had macroscopic alterations and

56% had alterations of the tendon microstructures, including the presence of inflammatory cells. It was also observed in their study that the rats that received corticoid lost weight, in comparison with the control group. This weight variation may have occurred in our study, although it was not evaluated in our animals. The parameters of their study diverged from that of ours, in that they evaluated the macro- and microscopic aspects of the tendons, while we evaluated the resistance of the healed tissue. In future studies, we will correlate our results with an evaluation of the tendon microstructure.

The tendons infiltrated with corticoid that were obtained from rats sacrificed one week after the repair showed significantly lower maximum tension values ( $\text{kgf}/\text{cm}^2$ ), in comparison with the controls. In the same group, the parameters of maximum force (kgf) and rupture force (kgf) demonstrated a tendency to also be lower, but the difference was not significant. Only Mikolyzk et al.<sup>15</sup> evaluated the influence of corticoid on tendons, at an early time of one week. In partially torn tendons, they observed a difference only in relation to maximum stress (MPa), without differences in maximum force (N) or in stiffness (N/mm). Despite the structural difference between these series, our results show some resemblance to those of Mikolyzk et al.<sup>15</sup>

In relation to tendons observed three weeks after corticoid infiltration, Mikolyzk et al.<sup>15</sup> did not demonstrate any decrease in resistance in any of the parameters analyzed. In the present study, we observed that the maximum tension value remained lower than in the control group, at the end of the third week.

There was no difference in the resistance of the infraspinatus tendons analyzed after five weeks, in comparison with the controls, either in our study or in the study by Mikolyzk et al.<sup>15</sup>

The present study has the limitation that it examined pure tendon injuries, which occur less frequently in rotator cuff injuries than in bone-tendon injuries. Another limitation of the study is that subacromial infiltrations are not routinely performed at the time of suturing but, rather, as treatment prior to the tendon repair, which thus restricts the extrapolation of the data to clinical practice.

Nonetheless, in evaluating the literature, we did not find any work similar to the present study that objectively assessed the influence of corticoids on the post-repair healing of the rotator cuff. Even so, previous studies demonstrated that there were worse clinical results in rotator cuffs repaired with repeated infiltrations. We consider that this is an important line of investigation to be followed in order to understand why there are worse results from rotator cuff repairs after repeated infiltrations.

## Conclusion

At the times of one and three weeks after suturing, the study group presented lower maximum tension in the healed tissue than that seen in the control group, but there were no differences in relation to maximum force and rupture force. Five weeks after the injury and suturing, the resistance of the healed tissue was equal regarding all parameters in the study and control groups.

## Conflicts of interest

The authors declare no conflicts of interest.

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