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Anabolic Steroids Reduce Muscle Damage Caused by Rotator Cuff Tendon Release in an Experimental Study in Rabbits

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**Background:** Muscles of the rotator cuff undergo retraction, atrophy, and fatty infiltration after a chronic tear, and a rabbit model has been used to investigate these changes. The purpose of this study was to test the hypothesis that the administration of anabolic steroids can diminish these muscular changes following experimental supraspinatus tendon release in the rabbit.

**Methods:** The supraspinatus tendon was released in twenty New Zealand White rabbits. Musculotendinous retraction was monitored over a period of six weeks. The seven animals in group I had no additional intervention, the six animals in group II had local and systemic administration of nandrolone decanoate, and the seven animals in group III had systemic administration of nandrolone decanoate during the six weeks. Two animals (group III) developed a postoperative infection and were excluded from the analysis. At the time that the animals were killed, in vivo muscle performance as well as imaging and histological muscle changes were investigated.

**Results:** The mean supraspinatus retraction was higher in group I (1.8 cm; 95% confidence interval: 1.64, 2.02 cm) than in group II (1.5 cm; 95% confidence interval: 1.29, 1.81 cm) or III (1.2 cm; 95% confidence interval: 0.86, 1.54 cm). Histologically, no fatty infiltration was measured in either treated group II (mean, 2.2%; range, 0% to 8%) or III (mean, 1%; range, 0% to 3.4%), but it was measured in the untreated group I (mean, 5.9%; range, 0% to 14.1%; \( p = 0.031 \)). The radiographic cross-sectional area indicating atrophy and the work of the respective muscle during one standardized contraction with supramaximal stimulation decreased in all groups, but the work of the muscle was ultimately highest in group III.

**Conclusions:** To our knowledge, this is the first documentation of partial prevention of important muscle alterations after retraction of the supraspinatus musculotendinous unit caused by tendon disruption. Nandrolone decanoate administration in the phase after tendon release prevented fatty infiltration of the supraspinatus muscle and reduced functional muscle impairment caused by myotendinous retraction in this rabbit rotator cuff model, but two of seven rabbits that received the drug developed infections.

**Clinical Relevance:** This study provides a novel approach that may have potential to diminish the irreparable structural and functional changes of the musculotendinous unit associated with chronic rotator cuff tear, but complications of anabolic steroid use also need to be considered.

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A commentary by Vincent M. Wang, PhD, is linked to the online version of this article at jbjs.org.
AFTER a rotator cuff tendon tear, the musculotendinous unit retracts and the muscle undergoesatrophy, fatty infiltration, and fibrosis with loss of contractility and elasticity. The torn tendon undergoes atrophy and structural disorganization. A tendonto-bone repair becomes increasingly difficult because of increased passive tension within the shortened musculotendinous unit and the poor tendon quality. If a repair is technically feasible, healing is uncertain. To date, full anatomical and functional restoration of a deteriorated musculotendinous unit has not been achieved. Continuous relengthening experimentally restored architectural changes caused by chronic rotator cuff tearing in a sheep model; fibrosis and atrophy of the muscle were halted and partially reversed by continuous traction and staged repair, but fatty infiltration could not be reversed either experimentally or clinically. New strategies for the prevention, or at least the reduction, of muscle degeneration are of substantial interest.

A patient may not always be aware of the onset of a rotator cuff tear. Even if such an event is known, surgery may not always be clinically necessary or practical. Prevention of muscle degeneration, however, always appears desirable to allow further treatment options to be successful. Pharmacologic prevention of muscle changes incurred by chronic retraction has not been investigated, as far as we know. There are substances that have an anabolic effect on muscle and could potentially counteract the formation of interferfiber gaps induced by atrophy, which are known to be filled with fat. Several pharmacological substances influence the development, growth, and repair mechanisms of muscle, namely, anabolic steroids, growth hormones, β-2 agonists, heparan sulfate, xanthine derivatives, creatine, and vitamin D. All of these substances, anabolic steroids seem best suited for clinical application. They enhance the synthesis of muscle proteins, induce hypertrophy of both type-I and II muscle fibers, and have an impact on satellite cell replication and activation and on contractile strength. Although their effect on treating established muscular alterations has been studied extensively, their effect on the prevention or reduction of deterioration of the muscle in a retracting musculotendinous unit is unknown.

The purpose of this study was to investigate whether anabolic steroids have a potential to lessen muscular changes caused by experimental musculotendinous retraction in a rabbit shoulder model.

**Materials and Methods**

**Animal Model and Groups**

This study was approved by the responsible investigational review board. A rabbit animal model was created: twenty New Zealand White rabbits with an average age of fifteen weeks and an average weight (and standard deviation) of 4.08 ± 0.42 kg underwent release of the supraspinatus tendon. Animals underwent preoxygenation through a face mask and received preoperative analgesia with intramuscular buprenorphine (10 μg per kilogram of body weight). Anesthesia was induced with medetomidine (5 μg/kg) and S-ketamine (7.5 mg/kg) intranasally before intubation was performed and inhalation anesthesia could be continued with isoflurane. After the right shoulder was shaved and disinfected, a skin incision of 5 cm was made parallel to the scapular spine and the supraspinatus tendon. The supraspinatus was visualized after dissection down to the deltoid muscle. Tendon release of the supraspinatus tendon was performed by osteotomy of the greater tuberosity. Intraoperative measurement of muscle strength as a function of different pretensions was performed in a standardized fashion both at the time of tendon release and at the time that the animal was killed. A force sensor (model 9203; Kistler, Winterthur, Switzerland) was connected to a fixator holding the sutures that grasped the tendon-bone chip complex. The fixator was stabilized by a firm connection to a sterilized rod with a threefold cup that was positioned at the acromion. The suprascapedular nerve was then stimulated with use of a needle electrode (Rochester Electro-Medical, Tampa, Florida) for supramaximal stimulation of the supraspinatus muscle with 20 mA and 40 Hz during 0.5 s. Stepwise release of the tension by 2 mm was allowed, starting at 10 N of passive pretension, and the force of the muscle was recorded before and during supramaximal stimulation during one contraction. The work was calculated by subtraction of the area under the curve of passive forces from the area under the curve of the maximal contractile forces over length.

Samples of the distal third of the supraspinatus muscle were harvested and fixed in formalin for further analysis before the tendon-bone chip complex was wrapped in a Penrose drain to prevent spontaneous healing, and the skin was closed. The musculotendinous unit was allowed to retract for six weeks without further intervention in seven animals (group I), with a weekly injection of nandrolone decanoate (50 mg/mL) partially locally into the supraspinatus muscle (0.4 mL locally and 0.8 mL into the quadriceps femoris muscle) in six animals (group II), or with total peripheral injection of nandrolone decanoate (0.6 mL each into the left and the right quadriceps muscle) in seven animals (group III) starting at the time of tendon release. In group II, only 0.4 mL of the substance was injected locally to diminish muscle changes caused by the oily carrier. An additional amount of 0.8 mL was injected systemically to achieve the same dosage as in group III with systemic application alone. Two animals from group III developed a postoperative infection at the site of the surgery and were killed four weeks after the initial surgery and excluded from the analysis. Postoperatively, the animals were allowed to walk freely, and they had a reoperation six weeks after the tenotomy to measure muscle work and to harvest biopsy specimens from both the operatively treated and the contralateral supraspinatus muscle before they were killed.

**Imaging and Histological Evaluations**

Computed tomographic (CT) measurements of retraction, atrophy, and fatty infiltration were performed both before and after tendon release with the rabbits in a lateral position. The measured retraction of the bone chip represented the amount of myotendinous retraction, the muscular cross-sectional area represented atrophy, and the density of the muscle, measured in Hounsfield units, represented fatty muscle infiltration and was determined with use of OsirisX software (version 3.6.1, 32 bit; http://www.osirisx-viewer.com). The retraction of the tendon was measured by the distance between the bone chip and the site of the osteotomy at the greater tuberosity in the transverse plane. The osteotomy site was projected on the image where the bone chip was most clearly identifiable for measurement of the direct distance (Fig. 1). The cross-sectional area was measured at the transition from the middle to the distal third (determined in the corresponding transverse plane) of the supraspinatus muscle in the sagittal plane and is reported in total values and as a percent of the contralateral, control side. All imaging measurements were repeated, with an intervening period of two months, by the same observer (M.F.), who was blinded to the type of treatment applied and the first reading of the results. The mean value of two corresponding measurements was used for analysis. For imaging measurements made at the time the animals were killed, the intraobserver reliability assessed by the Pearson correlation was highest for the assessment of fatty infiltration ($r^2 = 0.81$), followed by measures of retraction ($r^2 = 0.75$), and was moderate for the determination of the cross-sectional area ($r^2 = 0.58$). After an animal was killed, the supraspinatus muscles of both shoulders were dissected and weighed. For histological and morphometric evaluation, samples were stained with hematoxylin–eosin. A fivefold magnification was achieved with the microscope to capture pictures as digital images (in tagged image file format [TIFF]) to document fiber diameter (Fig. 2) on hematoxylin–eosin-stained sections. In addition, the proportions of fat, muscle, fibrosis, and background were analyzed by means of
histomorphometry by a blinded observer, as described previously\textsuperscript{5,10}, by coloring four different phases with a software program (Adobe Photoshop 7; Adobe Systems, San Jose, California). The background, representing empty space on the histological sections, was subtracted before calculation of the proportional amounts of fat, muscle, and fibrosis. Fiber diameter was measured by defining a line that was perpendicular to the longitudinally cut muscle fibers that served as a guide for the measurement of ten consecutive and clearly identifiable fiber diameters, starting always at the lower right end of the image.

**Statistical Analysis**

For statistical analysis, the software GraphPad Prism (version 4; GraphPad Software, La Jolla, California) was used. Grouped data were tested for normal
distribution with use of the Kolmogorov-Smirnov test before the use of either the Pearson correlation for Gaussian population or the Spearman correlation for nonparametric data. Intragroup comparison was performed with use of the two-tailed paired Student t test or Wilcoxon matched pairs test for normally distributed and not normally distributed data, respectively. Intergroup comparison was performed with use of the two-tailed paired Student t test or the Mann-Whitney test for normally distributed and not normally distributed data, respectively, and for both comparisons of different groups or intersample comparisons of the treated and the contralateral side. Values are reported as the mean and the standard deviation or as the range (minimum to maximum), when applicable. The level of significance was set at p < 0.05.

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The experiments and data analysis within this study were funded by the Balgrist Stiftung.

**Results**

**Work of the Muscle**
The work of the muscle during one standardized supramaximal stimulation decreased in all groups. The decrease was from 1.59 Nm (95% CI: 1.3, 1.88 Nm) to 1.2 Nm (95% CI: 0.99, 1.44 Nm; p = 0.056) and group II (mean, 2.06 Nm [95% CI: 1.30, 2.82 Nm] to 0.9 Nm (95% CI: 0.68, 1.13 Nm; p = 0.052) but less in group III (mean, 2.2 Nm [95% CI: 1.36, 3.04 Nm] to 1.6 Nm [95% CI: 0.69, 2.48 Nm]; p = 0.23). The error bars indicate the standard error of the mean. **Fig. 3** The mean amount of retraction of the musculotendinous unit of the supraspinatus was highest in group I (1.8 cm; 95% CI: 1.64, 2.02 cm) followed by group II (1.5 cm; 95% CI: 1.29, 1.81 cm) and group III (1.2 cm; 95% CI: 0.86, 1.54 cm) and was significantly higher in group I than in group II (\*p = 0.044) or III (\**p = 0.001). Box plots are depicted with whiskers from minimum to maximum. The horizontal line in the middle of each box represents the median value, and the top and bottom borders of the box represent the 75th and the 25th percentile, respectively.

**Imaging Measurements**
The amount of retraction was highest for group I (1.8 cm; 95% CI: 1.64, 2.02 cm), followed by group II (1.5 cm; 95% CI: 1.29, 1.81 cm) and group III (1.2 cm; 95% CI: 0.86, 1.54 cm) (Fig. 4), and was significantly higher in group I than in group II (\p = 0.044) or group III (\p = 0.001). There was a significant decrease of Hounsfield units (H) as an indicator of fatty infiltration on the CT images, from 62 H (95% CI: 60.1, 63.2 H) to 43 H (95% CI: 34.5, 51.7 H) in the untreated animals (group I; \p = 0.002), a moderate decrease in group II (57 H [95% CI: 53.4, 61.0 H] to 49 H [95% CI: 41.4, 57.5 H]; \p = 0.089), and no apparent change in group III (67 H [95% CI: 59.1, 74.3 H] to 65 H [95% CI: 58.2, 72.2 H]; \p = 0.76) (Fig. 5). The cross-sectional area set in relation to the contralateral side, measured on sagittal CT images, decreased from 107% (95% CI: 96%, 117%) to 82% (95% CI: 73%, 91%) in group I, and from 101% (95% CI: 97%, 107%) to 89% (95% CI: 84%, 94%) in group II, and from 99% (95% CI: 95%, 103%) to 85% (95% CI: 80%, 91%) in group III (Fig. 6). The error bars represent the standard error of the mean.
discussed after chronic retraction in the untreated animals (group I) (groups II and III), whereas significant fatty infiltration occurred in the pharmacologically treated animals six weeks of retraction (see Appendix). No significant fatty infiltration was noted in the untreated animals in group I (mean, 0.3% [range, 0% to 1.1%] to 5.9% [range, 0% to 14.1%]; p = 0.031*). The error bars represent the standard error of the mean. Fig. 7 Muscle fiber diameter decreased most after tendon release in group I (mean, 65 μm [95% CI: 56, 75 μm]) to 48 μm [95% CI: 33, 64 μm]; p = 0.063) and remained similar in the treated groups II (mean, 60 μm [95% CI: 52, 68 μm]) to 67 μm [95% CI: 48, 85 μm]) and III (mean, 63 μm [95% CI: 53, 72 μm]) to 55 μm [95% CI: 24, 86 μm]; p = 0.640). The error bars indicate the standard error of the mean.

Discussion

The structural and functional alterations of the musculotendinous unit as a consequence of chronic tendon tears are considered irreversible on direct tendon repair and have been shown to be partly reversible with continuous re-lengthening of the retracted myotendinous unit[10]. It is desirable to inhibit the development of these changes in the first place. If a surgical repair cannot be performed shortly after a supraspinatus tear, a pharmacological approach to inhibit muscle degeneration could be valuable. We documented inhibition of fatty infiltration and partial preservation of muscle function by administration of nandrolone decanoate during musculotendinous retraction in a rabbit rotator cuff tear model. These experimental observations suggest a new approach with potential for preventing tendon-tear-induced muscle deterioration.

This study has some limitations. First, this is an animal study and translation of these findings to the human should be made with caution. The rabbit rotator cuff model is well accepted for investigation of pathologic conditions of muscle because it reproduces the key structural findings for muscle deterioration seen in humans and sheep after detachment of its tendon[10-12]. This study focused exclusively on the muscle tissue and did not investigate the tendon, primarily because...
the available amount of tendon tissue was insufficient for a biopsy-based analysis. The rat model has been proposed for studies of pathological conditions of the supraspinatus tendon on the basis of the similarity of the subacromial anatomy to the human shoulder. So far, investigators have failed to quantitatively satisfactorily reproduce the characteristic muscular changes seen in humans and sheep with use of the rat model. The sheep model is well established for investigations of muscular and tendinous rotator cuff abnormality. Although investigations in which technical feasibility (e.g., implantation of a device to continuously relengthen retracted myotendinous units) favors large-animal models, we found the current smaller-animal model suitable to answer the research questions at hand while reducing the cost and ethical burden. Although the fatty infiltration observed in the rabbit rotator cuff model is multiple times lower than levels seen in the human, the model provided sufficient sensitivity to assess the effectiveness of the pharmacological intervention.

The optimal time allowed for retraction and development of muscular changes in the rabbit rotator cuff model is controversial: Björkenheim documented peak alteration after six weeks of retraction, and other authors have suggested the possibility for further progression. It was not the purpose of this study to investigate the evolution of muscular changes over time, but to study the animals before and after a substantial muscular alteration had been induced by the release of the supraspinatus tendon and consequent musculotendinous retraction. Significant deterioration of the detached muscle was observed within six weeks of retraction in the untreated group, and substantial differences between the control and test groups were identified. We believe that proof of principle was sufficiently established and that the original hypothesis was confirmed.

Second, potentially detrimental side effects of the applied anabolic steroid were not investigated. Two animals with systemic application of nandrolone decanoate developed wound infections, and we do not know whether the steroid was causative. Further, translation of our findings to humans regarding potential beneficial effects of nandrolone decanoate needs further research. The effect of anabolic steroids on striated muscle is acceptance as unavoidable and irreversible opens new research potential. Other growth hormones and myogenic stimulators should be tested further, particularly as optimal dosage has not been addressed. Further, potential adverse effects on the tendinous tissue as well as systemic adverse effects of anabolic steroids should be considered.

Appendix

A table showing muscle, fat, and fibrotic tissue as a percentage of total tissue according to histomorphometric measurement at tenotomy and after six weeks of chronic musculotendinous retraction is available with the online version of this article as a data supplement at jbjs.org.

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