Substantial Biomechanical Improvement by Extracorporeal Shockwave Therapy After Surgical Repair of Rodent Chronic Rotator Cuff Tears

Xaver Feichtinger,*[†‡§||] MD, PhD, Xavier Monforte,[§¶] MSc, Claudia Keibl,[†§] DVM,
David Hercher,[†§] MSc, Jakob Schanda,[†‡§] MD, Andreas H. Teuschl,[§¶] PhD,
Christian Muschitz,[#] MD, Heinz Redl,[†§] PhD, Christian Fialka,[‡**] MD, MBA,
and Rainer Mittermayr,[†‡§] MD
*Investigation performed at Ludwig Boltzmann Institute
for Experimental and Clinical Traumatology, Vienna, Austria*

Background: Characteristics of chronic rotator cuff tears include continuous loss of tendon structure as well as tendon elasticity, followed by a high failure rate after surgical reconstruction. Several studies have already shown the beneficial effect of extracorporeal shockwave therapy (ESWT) on tissue regeneration in tendon pathologies.

Hypothesis: ESWT improves biomechanical tendon properties as well as functional shoulder outcomes in chronic rotator cuff reconstruction in rodents.

Study Design: Controlled laboratory study.

Methods: After tendon detachment and 3 weeks of degeneration, a subsequent transosseous reattachment of the supraspinatus tendon was performed in 48 adult male Sprague-Dawley rats (n = 16 per group). Rodents were randomly assigned to 3 study groups: no ESWT/control group, intraoperative ESWT (IntraESWT), and intra- and postoperative ESWT (IntraPostESWT). Shoulder joint function, as determined by gait analysis, was assessed repeatedly during the observation period. Eight weeks after tendon reconstruction, the rats were euthanized, and biomechanical and gene expression analyses were performed.

Results: Macroscopically, all repairs were intact at the time of euthanasia, with no ruptures detectable. Biomechanical analyses showed significantly improved load-to-failure testing results in both ESWT groups in comparison with the control group (control, 0.629; IntraESWT, 1.102; IntraPostESWT, 0.924; IntraESWT vs control, $P \le .001$; IntraPostESWT vs control, $P \le .05$). Furthermore, functional gait analyses showed a significant enhancement in intensity measurements for the IntraPostESWT group in comparison with the control group ($P \le .05$). Gene expression analysis revealed no significant differences among the 3 groups.

Conclusion: Clearly improved biomechanical results were shown in the single-application and repetitive ESWT groups. Furthermore, functional evaluation showed significantly improved intensity measurements for the repetitive ESWT group.

Clinical Relevance: This study underpins a new additional treatment possibility to prevent healing failure. Improved biomechanical stability and functionality may enable faster remobilization as well as an accelerated return to work and sports activities. Furthermore, as shockwave therapy is a noninvasive, easy-to-perform, cost-effective treatment tool with no undesired side effects, this study is of high clinical relevance in orthopaedic surgery. Based on these study results, a clinical study has already been initiated to clinically confirm the improved functionality by ESWT.

Keywords: chronic rotator cuff tear; ESWT; shockwave; biomechanical analysis

Rotator cuff tears are frequent orthopaedic disorders with challenging treatment circumstances.[29,44] Recurrent postoperative defects are seen consistently and can reach up to 94.4% in massive rotator cuff tears.[6,12,36] Limiting factors for rotator cuff healing after surgical repair are size of the original tear, tear chronicity, and patient age.[6,12,13] Fatty infiltration of the muscles, muscle atrophy, muscle and tendon retraction, fibrosis, and low bone quality, including bone microstructure and bone mineral density, are characteristics of degenerative chronic tears.[8,20,27] Preclinical studies are focused particularly on acute tears and healing outcomes. In contrast to traumatic rotator cuff

tears, healing processes and characteristics are poorly understood in chronic degenerative rotator cuff tears.[19,20]

Extracorporeal shock wave therapy (ESWT) has been in clinical use since the 1980s.[5] Extracorporeally generated shock waves are introduced into the body without injuring the skin. The beneficial effect of ESWT on tissue regeneration has been demonstrated in numerous experimental and clinical studies.[7,40,42] Particularly with respect to tendon tissues, ESWT increases collagen synthesis of tenocytes and decreases expression of interleukins and matrix metalloproteases, which are associated with tendinopathy.[38] In tendon and muscle tissues, improved blood flow was shown after a single application of ESWT, which was prolonged by repetitive ESWT application.[21]

Given the high failure rate after rotator cuff reconstruction, improvements in treatment strategies are in great demand. To our knowledge, there are currently no studies preclinically investigating the effect of ESWT on the healing of surgically treated chronic rotator cuff tears.

The main aim of this study was to determine the effect of ESWT on the biomechanical outcome in experimental chronic rotator cuff tears. Additionally, the study evaluated the effect on functional outcome and gene expression.

The main hypothesis was that a single ESWT application improves the biomechanical as well as functional outcome of surgically treated chronic rotator cuff tears.

METHODS

Animal Model

Male Sprague-Dawley rats (n = 48) were used, as approved by the Institutional Animal Care and Use Committee. The acclimatization period was fulfilled with 1 week. Rats were housed in a temperature- and light-controlled room in groups of 2 (12-hour light-dark cycle). Surgery was performed according to a randomization protocol dedicated to 3 study groups (n = 16 rats per group): control/no ESWT, intraoperative shockwave therapy (IntraESWT), intraoperative and postoperative shockwave therapy (IntraPostESWT) (Figure 1). Age and weight of the rats were homogeneous in the 3 groups (400-410 g). At time point zero, all rats underwent unilateral tenotomy of the supraspinatus (SSP) muscle of the left shoulder under general anesthesia. An incision was made on the anterolateral aspect of the left shoulder, and a deltoid muscle split was performed.[37] After surgical

exposure of the SSP tendon, sharp detachment from the bone at the insertion area was carried out.[20] Because of adhesion formation and organized scar tissue, the SSP tendon was clasped with a suture and left inside during the detachment operation.[4] The deltoid muscle was readapted, and the skin incision was closed with Vicryl 4-0 suture (Ethicon Inc). Three weeks after the induced injury, the left side was reoperated, and tendon repair was conducted with a modified Mason-Allen stitch (Prolene 5-0 suture; Ethicon Inc) and transosseous sutures. Thereby, the insertion area was debrided gently, and a bone tunnel was drilled in an anteroposterior direction close to the greater tuberosity.[20] The suture was passed through the bone tunnel and secured to readapt the tendon end to its footprint.[20] Readaption and closure of the skin were performed as in the primary operation. Subsequently, intraoperative shockwave therapy was performed in the IntraESWT and IntraPostESWT groups. In total, 600 impulses were applied unfocused on the insertion side by an electrohydraulic generation mechanism with an energy flux density of 0.19 mJ/mm^2 at 3 Hz (DermaGold; Tissue Regeneration Technologies, LLC; manufactured by MTS Europe GmbH). Pain treatment was conducted in all animals equally, and free cage activity with an enriched environment was allowed. One week postoperatively, animals in the IntraPostESWT group received another ESWT performed equally to the first application with the same electrohydraulic device, energy flux density, and number of impulses (Figure 1). Eight weeks after tendon reattachment, the animals were sacrificed in deep anesthesia by an overdose of thiopental intracardially. Both humeri were then exarticulated by gentle exposure and careful preparation without any delay. Care was taken to maintain the SSP tendon attachment. The other tendons were removed carefully. Small soft tissue remains around the humeral head were left untouched for tendon and bone protection (Figure 2A). One rat, belonging to the control group for gene expression analysis and gait analysis, died after the second surgical intervention owing to perioperative anesthesiologic complications. Another rat, belonging to the IntraPostESWT group, was excluded from the study for joint infection (Figure 1).

Biomechanical Analysis

Humeral specimens were stored in 1% penicillin-streptomycin solution (Aqua Bidest) at 4°C until biomechanical testing was performed within 48 hours. Biomechanical testings were performed in 36 rats in the

*Address correspondence to Xaver Feichtinger, MD, PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Donaueschingenstraße 13, 1200 Vienna, Austria (email: xaver.feichtinger@gmail.com).

†Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria.
‡AUVA Trauma Center Vienna–Meidling, Vienna, Austria.
§Austrian Cluster for Tissue Regeneration, Vienna, Austria.
‖Department of Orthopaedic Surgery II, Herz-Jesu Krankenhaus, Vienna, Austria.
¶Department of Life Science Engineering, University of Applied Sciences Technikum Wien, Vienna, Austria.
#Metabolic Bone Diseases Unit, St Vincent Hospital, Vienna, Austria.
**Center for the Musculoskeletal System, Medical Faculty, Sigmund Freud University, Vienna, Austria.
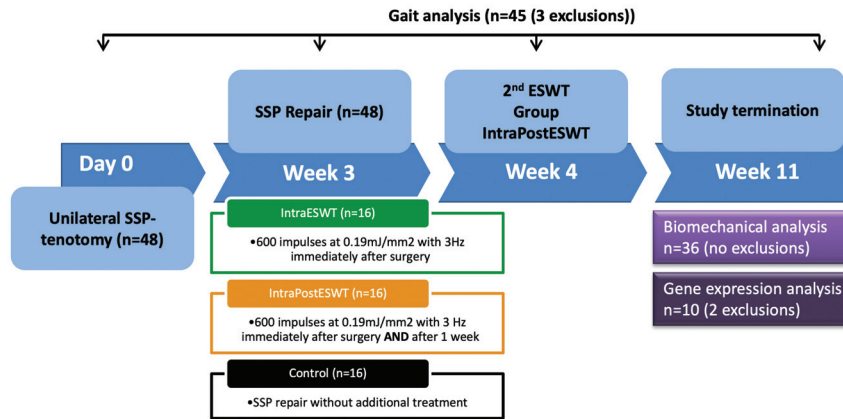
Figure 1. Schematic representation of the study design. ESWT, extracorporeal shockwave therapy; IntraESWT, intraoperative ESWT; IntraPostESWT, intra- and postoperative ESWT; SSP, supraspinatus.
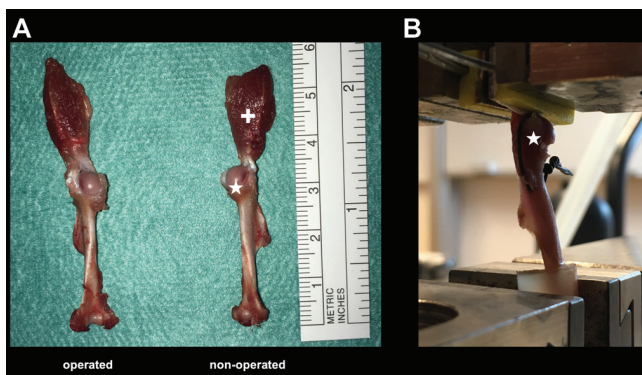

Figure 2. (A) Exarticulation and preparation of the humeri, including the SSP tendon immediately after sacrifice (left: operated side, right: nonoperated side). (B) Biomechanical testing: fixation of the sample in a 10° angle with the distal section of the humerus potted in polymethyl methacrylate and the SSP tendon fixed in a sand paper clamp. ★, humeral head; +, SSP tendon. SSP, supraspinatus.

surgically treated groups as well as their contralateral sides. Testing was carried out by 1 investigator (X.F.) blinded concerning the study group. To assess only regenerated tissue, sutures of the tendon reattachment were discarded before testing was performed. As described in earlier studies, a hole was drilled through the humerus diaphysis, and a steel wire was passed through and wrapped around the humeral head to prevent growth plate failure.[20] A small notch at the superior location of the humeral head was created with a file to hold the steel wire in position during biomechanical testing. The distal section of the humerus was potted in polymethyl methacrylate, and to prevent slippage, the SSP tendon was fixed in a sand paper clamp as close as possible to the tendon footprint (Figure 2B). Testing was performed at room temperature (21°C). In accordance with a previous study, specimens were fixed pneumatically in a 10° angle (corresponding 80° abduction)

in a uniaxial testing machine (BZ 2.5/TN1S; Zwick/Roell)[30] (Figure 2B). Preconditioning was performed by applying 5 preload cycles with a 0.6-N load and a 0.2-N resting phase with 30-second hold times for both. After preconditioning, the specimens were tested until failure with a constant testing speed of 0.5 mm/min. The force was recorded with TestXpert Software (Zwick/Roell), and the maximum force was used for comparison among the groups.

Gait Analysis

Gait analysis was performed with CatWalk XT (v 10.5; Noldus Information Technology) in all study rats (n = 45; 2 exclusions as mentioned earlier, plus 1 additional exclusion from the IntraPostESWT group because of technical problems) at 4 time points: preoperatively (baseline), 1 day before the second operation, 2 weeks after the second operation, and 8 weeks after the second operation before euthanasia. Owing to a habituation process, training runs were performed in all rats. All analyses were conducted by 2 experienced examiners blinded regarding the study group. The CatWalk system consists of a glass runway and allows visualization of individual prints of the crossing animals as well as information regarding speed and consistency of the ambulation. A video camera records the locomotion of the animal.[10] A mean of at least 3 runs consisting of 3 representative prints of every paw were used for analysis.[23] Gait alterations were detected by the use of results of the left front paw, expressed as a ratio of the right front paw. The following parameters were measured: print area, mean intensity, maximum contact–mean intensity, swing duration, and stride length.

Gene Expression

The surgically treated shoulder and the contralateral side of 10 rats (owing to 2 exclusions as described earlier; Intra-ESWT, n = 4; IntraPostESWT, n = 3; control, n = 3) underwent gene expression profiling. After exarticulation of the

TABLE 1
Primer Sequences for Gene Expression Analyses
With Annealing Temperatures and Base Pairs[a]

Primer	Sequence	Base Pairs	Annealing Temperature, °C
CXCL12			58
s	GAG CCA ACG TCA AAC ATC TG	20	
as	GTT TAA GGC TTT GTC CAG GT	20	
TGF-β1			60
s	CAACAATTCCTGGCGTTACC	20	
as	GACTGATCCCATTGATTTCCA	21	
TGF-β3			60
s	GCTCTTCCAGATACTTCGACCT	22	
as	GATGCTGATTTCCAGACCCA	20	
VEGFR2			51
s	GGA TTT ATG ATG TGG TCC TG	20	
as	CTT ATG CTG ATG CTT TGA GG	20	
HPRT			63.5
s	AGT CCC AGC GTC GTG ATT AG	20	
as	TGG CCT CCC ATC TCC TTC AT	20	

[a]as, antisense primer; CXCL12, C-X-C motif chemokine 12; HPRT, hypoxanthine guanine phosphoribosyltransferase; s, sense primer; TGF-β1, transforming growth factor beta 1; TGF-β3, transforming growth factor beta 3; VEGFR2, vascular endothelial growth factor receptor 2.

humerus and careful separation of the SSP muscle, a muscle sample was extracted without tendon tissue of the SSP middle third. The samples were extracted at the same muscle location in each rat. They were placed in an empty microcentrifuge tube, shock-frozen by liquid nitrogen, and put into a freezer cooling to –80°C. Homogenization and RNA purification were performed to isolate RNA. Measurement of RNA concentration was performed by spectrophotometry. Yield and purity (A260/A280) of RNA (NanoDrop OneC; Thermo Scientific) was checked, and DNA digestion and RNA precipitation were conducted for every sample. RNA (2 μg) was used to perform cDNA synthesis with a OneScript (ABM) cDNA syntheses kit. Quantitative reverse transcription polymerase chain reaction was carried out with 40 ng of cDNA and the use of a CFX96 detection module and a C1000 thermal cycler (Bio Rad) for preparation. CFX Manager Software (Bio Rad) was used for analysis. *CXCL12* (C-X-C motif chemokine 12), *TGF-β1* and *TGF-β3* (transforming growth factor beta 1 and 3), and *VEGFR2* (vascular endothelial growth factor receptor 2) expression was measured (Table 1). The reference gene *HPRT* (hypoxanthine guanine phosphoribosyltransferase), as a known reference gene for inflammatory and healing failure,[22,39] was used to normalize gene expression measurements.

Statistical Analysis

Power analysis was performed with the primary outcome of load to failure (load$_{max}$, N) in biomechanical analysis based on a previous study that evaluated rotator cuff tendon healing.[14] With these estimations, a power of 0.80 is achieved with 12 specimens per group (α = .05). Testing for normal distribution was performed with the D'Agostino and Pearson omnibus normality test. In case of normal

TABLE 2
Biomechanical Analysis: Load to Failure[a]

	Load to Failure[b]		
	No ESWT	Intra ESWT	IntraPost ESWT
Mean	0.629	1.102	0.924
SD	0.24	0.30	0.20

	Group Differences	*P* Value[c]
IntraESWT vs no ESWT	0.474 ± 0.11	≤.001
IntraPostESWT vs no ESWT	0.295 ± 0.09	≤.05
IntraPostESWT vs IntraESWT	–0.178 ± 0.10	ns

[a]ESWT, extracorporeal shockwave therapy; IntraESWT, intraoperative ESWT; IntraPostESWT, intra- and postoperative ESWT; ns, not significant.
[b]Operated forepaw expressed as a ratio of the healthy forepaw.
[c]One-way analysis of variance and Tukey multiple-comparisons test.

distribution, 1-way analysis of variance and Tukey/Sidak multiple-comparisons tests were conducted. In cases of no normal distribution, the Dunn multiple-comparison test and Kruskal-Wallis test were carried out. Results of the surgically treated side were compared with the contralateral side and expressed as a ratio thereof. Within the groups, paired *t* tests were used in the case of normal distribution. GraphPad Prism (v 6.00; GraphPad Software) was used for statistical calculations of results and creation of graphs.

RESULTS

SSP muscle and tendon structure showed no macroscopic differences among the groups after euthanasia. No gap formations at the tendon footprint and no suture disruptions were detectable (Figure 2).

Biomechanical Analysis

Biomechanical analysis was performed on all 36 rats for the surgically treated and contralateral sides. Both ESWT groups showed significantly higher load-to-failure results than the control group (Table 2, Figure 3). Among the ESWT groups, no statistically significant difference was detectable. Comparisons within each group between the operated and healthy sides presented a significant difference in the control group. Here the operated side resulted in clearly lower load-to-failure outcomes than the nonoperated side (mean ± SD, 0.629 ± 0.24). In both ESWT groups, no significance between the surgically treated and healthy sides was detectable (IntraESWT, 1.102 ± 0.30; IntraPostESWT, 0.924 ± 0.20). The load-displacement curves for the operated joints showed a different profile as compared with the contralateral joints, with a distinct toe region.
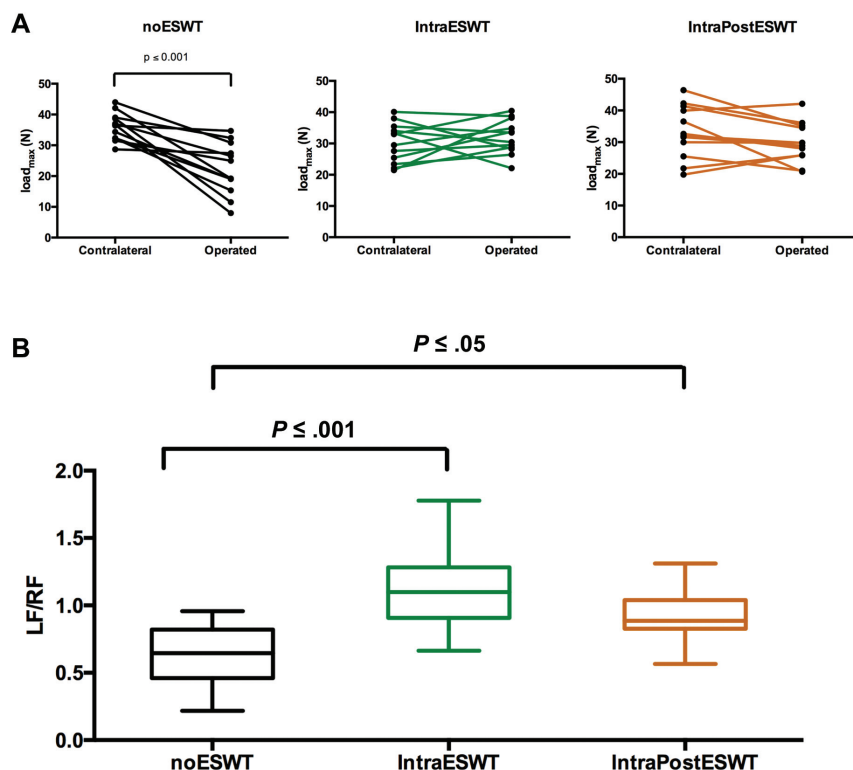
Figure 3. (A) Load-to-failure testing (load maximum, N) within each group, reaching a significant difference in the noESWT group (paired *t* tests). (B) Load-to-failure results as a ratio of the operated left forepaw (LF) to the nonoperated right forepaw (RF). Values are presented as median, interquartile range, and minimum/maximum. *P* values were lined out in case of significance (1-way analysis of variance and Tukey multiple-comparisons test). ESWT, extracorporeal shockwave therapy; IntraESWT, intraoperative ESWT; IntraPostESWT, intra- and postoperative ESWT.

The treatment groups, both IntraESWT and IntraPostESWT, showed no significant difference regarding loading curves in comparison with the untreated joints. The healthy joints showed a more continuous profile, with less distinctive ruptures during the test when compared with the operated joints (Figure 4).

Gait Analysis

CatWalk XT gait analysis was performed in 45 rats. No significant differences among the study groups were detectable for the parameters of print area, swing duration, and stride length (see Figure 5). Eight weeks after reattachment surgery (late rehabilitation time), analyses for the intensity parameters presented a rising curve in both ESWT groups, reaching significance in mean intensity (adjusted $P \leq .05$) (Figure 5).

Gene Expression in the Affected SSP Muscle

Gene expression profiling was performed in 10 rats. In TGF-β3 measurements, 1 sample from the IntraESWT group had to be excluded for technical issues. No significant differences

were found among the 3 groups for each gene expression measurement (Table 3).

DISCUSSION

The aim of this study was to determine the effect of ESWT on the biomechanical outcome in experimental chronic rotator cuff tears. Furthermore, we chose to evaluate its effect on shoulder function and gene expression.

Significantly improved biomechanical results were detected in ESWT-treated groups after surgical repair in this chronic rotator cuff tear model. Significantly increased load to failure was shown in both the IntraESWT group and the IntraPostESWT group. Additionally, shoulder function evaluated by gait analysis showed improvement in ESWT groups reaching significance in the mean intensity parameter.

Since high failure rates after reconstruction are associated with tear chronicity and tear size, understanding the healing mechanisms and improving therapy strategies in chronic rotator cuff tears are of significant value.[20]

Literature dealing with shockwave treatment in tendon repair is rare. To date, the central mechanisms of ESWT have been investigated and biomolecular and cellular
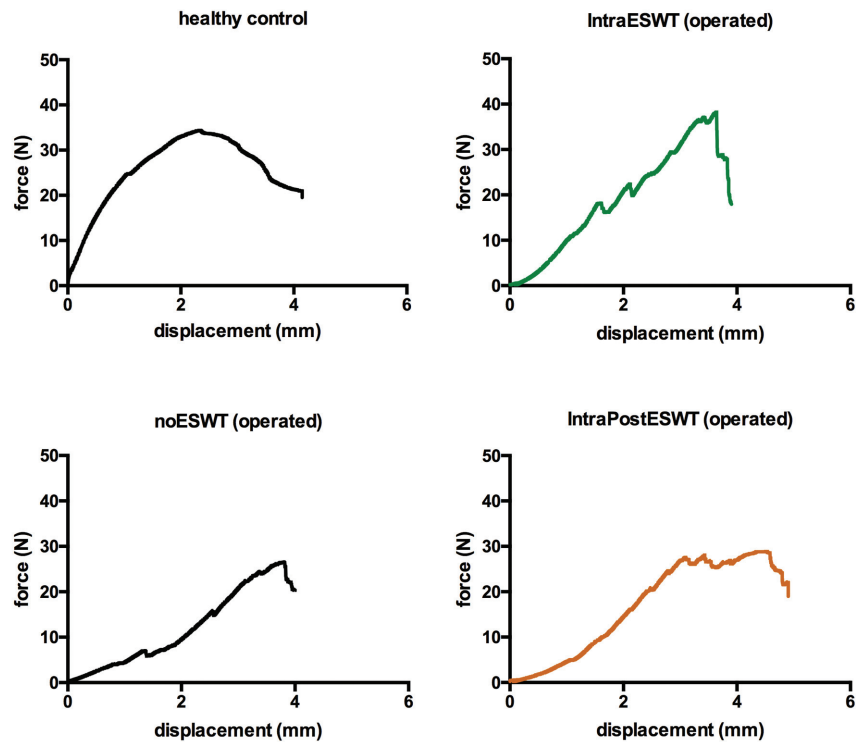
Figure 4. Biomechanical analysis: representative load-to-failure curves (force, N) of each study group in comparison with a healthy control. ESWT, extracorporeal shockwave therapy; IntraESWT, intraoperative ESWT; IntraPostESWT, intra- and postoperative ESWT.

processes described, in which purinergic signaling via the ERK1/2 pathway and subsequent overexpression of various growth factors seem to play an especially key role.[26,28,42,46] Vascular endothelial growth factor—known to induce angiogenesis, lymphangiogenesis, and collateral vessel formation—and nitric oxide as a potent vasodilator were shown to be induced by ESWT, in addition to inflammatory modulation.[3,15,25,32,46] In an acute Achilles tendon injury model, Orhan et al[31] found better vascularization as well as a greater force required to rupture the tendon after application of shockwave treatment. Further important mechanisms appear to be an increase of collagen synthesis and cell growth, as well as a decreased expression of interleukins and matrix metalloproteases.[38] Kisch et al[21] focused on repetitive ESWT and described additional benefits on blood flow and vascularization in physiological tissue without inducing any injuries. There are no studies investigating the effect of single or repetitive ESWT on healing after tendon reconstruction in chronic tendon tears.

Buchmann et al[4] suggested that a 3-week period after defect in rodents was comparable to the degenerative situation in humans, owing to the high self-healing potential of rats as well as the high risk of fatty infiltration in humans. The effect of footprint preparation techniques on the outcome of rotator cuff reconstruction was investigated in earlier studies.[2,11,34] Based on these results, debridement of the SSP tendon insertion area was performed in this study.

In this study, clearly improved biomechanical results were shown in both shockwave-treated groups in comparison with the control group (Figure 3). Interestingly, the IntraESWT group achieved better results than the IntraPostESWT group, the reasons for which have yet to be fully clarified. A distinct possibility to account for these results could be that additional anesthesia in the IntraPostESWT group slowed the rehabilitation process, although there were no differences in obvious general behavior among the 3 study groups.

Gait analysis was performed as described in earlier studies.[18,43,45] High homogeneity among the 3 groups and within each group was shown for the print area parameter. Swing duration—as a reliable parameter for functional evaluation in neurorehabilitation—showed no significant differences among the groups.[18] Stride length measurements revealed longer distances in the control group shortly after operation. But long-term investigations (8 weeks) showed longer distances, without reaching significance, in the ESWT groups. Mean intensity as well as maximum contact—mean intensity measurements were improved in both ESWT groups in comparison with the control group with no ESWT. Eight weeks after tendon reattachment, significant improvement was detected for the IntraPostESWT group (Figure 5). As these results are shown in the long-term observation period (8 weeks after the second operation before euthanasia), it seems not to be due to pain release by shockwave treatment in
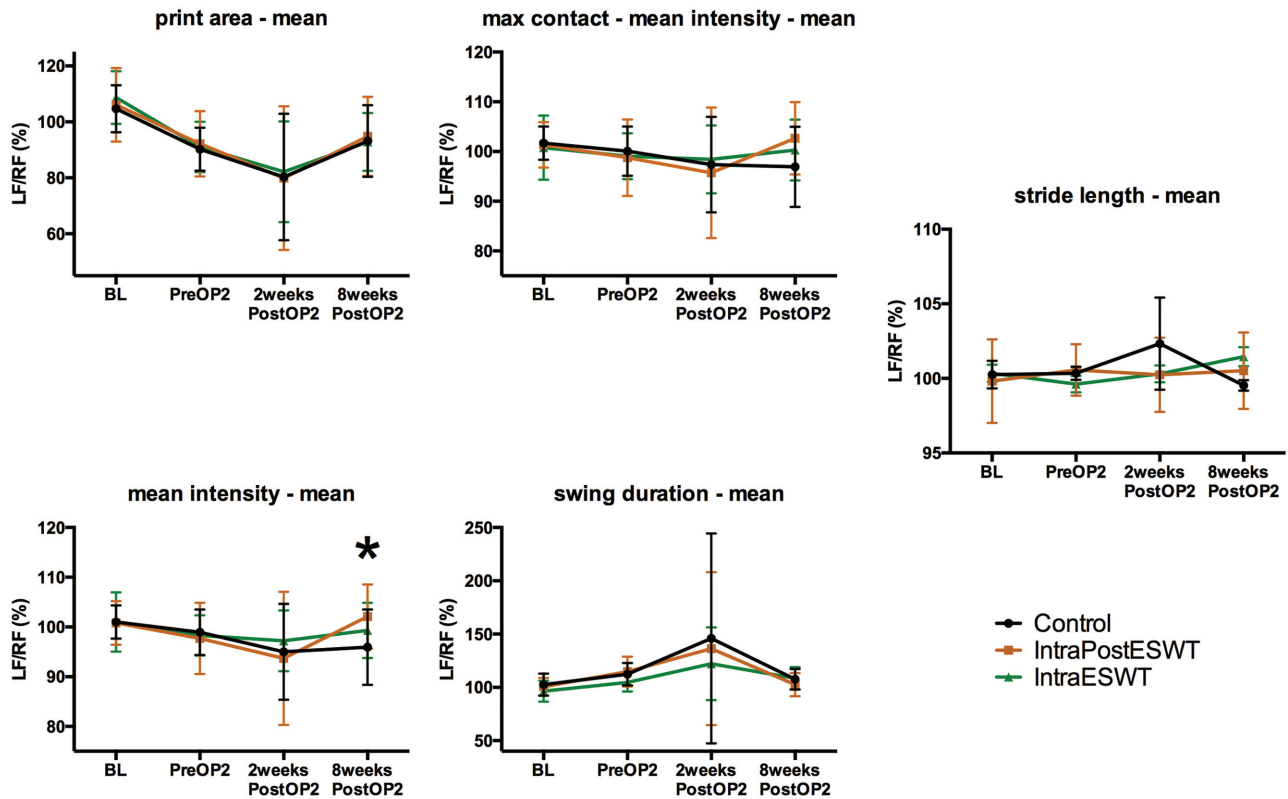
Figure 5. Mean and SD at baseline (BL), 1 day before the second operation (PreOP2), 2 weeks after the second operation (2weeksPostOP2), and 8 weeks after the second operation (8weeksPostOp2). LF/RF (%): ratio of the operated left forepaw (LF) to the nonoperated right forepaw (RF). *$P < .05$, control group vs IntraPostESWT group.

the ESWT groups. Improvement of shoulder function is multifactorial. An important factor seems to be tendon attachment strength. In humans, functional improvement—especially regarding early tendon attachment strength—would be helpful to reduce the rate of recurrent tendon detachment, as it is often seen in the early months after active mobilization of the shoulder.

CXCL12 and VEGF-R2 play important roles in angiogenesis and were shown to be increased by ESWT in earlier studies.[16,28,35] Additionally, muscle regeneration is described to be positively influenced by CXCL12 through STAT3 signaling.[24] Interestingly, in this study, angiogenesis was not significantly improved in shockwave groups as detected by these parameters. As the tendon rupture was reconstructed in this model, mechanical properties of the rotator cuff were restored. At 8 weeks after reconstruction, the rehabilitation period appears to be completed regarding these parameters. However, as mentioned in earlier studies, neovascularization, vasodilation, and inflammatory modulation seem to play a key role in tendon regeneration by ESWT. The effect is assumed to occur in an earlier phase of tendon regeneration in rat models.[41] The TGF-β1:TGF-β3 ratio was lower in the shockwave-treated groups in this study. TGF-β1 is known to be more scar mediated and occurs in adult wound healing. In contrast, TGF-β3 is considered more regenerative than reparative.[40] Smad3 and Smad7 proteins were described to be regulated

TABLE 3
Gene Expression Analysis[a]

	No ESWT	IntraESWT	IntraPostESWT
VEGFR2			
Mean	0.61	1.12	0.87
SD	0.47	0.76	0.08
CXCL12			
Mean	0.73	1.64	1.41
SD	0.34	1.40	0.44
TGF-b1			
Mean	1.21	1.28	1.53
SD	0.62	0.81	1.19
TGF-b3			
Mean	1.98	2.12	1.07
SD	2.07	1.72	0.39
TGF-b1/TGF-b3			
Mean	3.89	0.64	1.08
SD	4.55	0.37	0.99

[a]Expressed as a ratio of the surgically treated side to the healthy side (Kruskal-Wallis and Dunn multiple-comparisons test). CXCL12, C-X-C motif chemokine 12; ESWT, extracorporeal shockwave therapy; HPRT, hypoxanthine guanine phosphoribosyltransferase; IntraESWT, intraoperative ESWT; IntraPostESWT, intra- and postoperative ESWT; TGF-β1, transforming growth factor beta 1; TGF-β3, transforming growth factor beta 3; VEGFR2, vascular endothelial growth factor receptor 2.

by TGF-β3 to minimize extrinsic scarring and improve muscle and tendon healing.[17] Despite basic differences in tendon healing and tendon-to-bone healing, these results may indicate a more regenerative healing process in the shockwave groups in contrast to a more scar-mediated process in the control group.

Limitations

A possible weak point of the present study seems to be the additional anesthesia in the IntraPostESWT group. Despite there being no detectable differences of cage activity among the groups, the additional anesthesia likely influenced the rehabilitation process. Given ethical concerns, it was not reasonable to perform additional narcosis in all study rats.

The age of the rats at the time of investigation, with open growth plates, may be another limitation. Because of the age- and health-related problems in older rodents with closed growth plates and for the reason of variability reduction, younger rats of like age with comparable health conditions were used, in keeping with other studies investigating chronic rotator cuff tears.[20]

The small sample size regarding gene expression analysis is a weak point of the present study. As a primary focus was set on biomechanical and functional analysis, study animals were divided throughout investigations accordingly. The lack of histological analysis is another possible limitation. Given the handling processes used in this study, histological evaluation of tendon healing was not possible. Based on the improved biomechanical results, a study focusing on histology and immunohistochemistry has already been initiated.

Another limitation may be the use of a rat model in this study design. Because of the different movement properties as well as weightbearing differences, results have to be interpreted with caution for translation to clinical practice. Despite these weaknesses, earlier studies described the rat as the most suitable animal model for investigation of basic mechanisms in tendon healing and postoperative activity levels in chronic rotator cuff tears.[1,9] Because of the long reparability of the tendons in rats and the similarity of bone and muscle anatomy to humans, rats provide decisive advantages.[33]

CONCLUSION

In conclusion, significantly improved load-to-failure tests were shown in both the single-application and repetitive ESWT groups in comparison with the control group. These results indicate that even a single ESWT application achieves beneficial effects and enhances the outcome. Furthermore, significantly improved intensity measurements were shown by using gait analyses for the IntraPostESWT group. Other functional parameters showed no significant differences among the groups.

As healing failure is indicated at a high rate after chronic rotator cuff reconstruction, this study underpins a new additional treatment possibility to prevent failure in humans—one that considers tendon quality, tear size, age, behavior, and tear chronicity. Improved biomechanical

stability may enable faster remobilization as well as a shorter time of return to work ability and sports activity. Furthermore, as shockwave therapy is a noninvasive, easy-to-perform, cost-effective treatment tool with no undesired side effects, this study is of high clinical relevance in orthopaedic surgery. Based on these laboratory study results, a clinical study has already been initiated to confirm the improvement by ESWT clinically.

REFERENCES

1. Barton ER, Gimbel JA, Williams GR, Soslowsky LJ. Rat supraspinatus muscle atrophy after tendon detachment. *J Orthop Res*. 2005;23(2):259-265.
2. Bonnevialle N, Bayle X, Faruch M, Wargny M, Gomez-Brouchet A, Mansat P. Does microvascularization of the footprint play a role in rotator cuff healing of the shoulder? *J Shoulder Elbow Surg*. 2015;24(8):1257-1262.
3. Brañes J, Contreras HR, Cabello P, Antonic V, Guiloff LJ, Brañes M. Shoulder rotator cuff responses to extracorporeal shockwave therapy: morphological and immunohistochemical analysis. *Shoulder Elbow*. 2012;4(3):163-168.
4. Buchmann S, Walz L, Sandmann GH, et al. Rotator cuff changes in a full thickness tear rat model: verification of the optimal time interval until reconstruction for comparison to the healing process of chronic lesions in humans. *Arch Orthop Trauma Surg*. 2011;131(3):429-435.
5. Chaussy C, Brendel W, Schmiedt E. Extracorporeally induced destruction of kidney stones by shock waves. *Lancet Lond Engl*. 1980;2(8207):1265-1268.
6. Chona DV, Lakomkin N, Lott A, et al. The timing of retears after arthroscopic rotator cuff repair. *J Shoulder Elbow Surg*. 2017;26(11):2054-2059.
7. Chow DHK, Suen PK, Fu LH, et al. Extracorporeal shockwave therapy for treatment of delayed tendon-bone insertion healing in a rabbit model: a dose-response study. *Am J Sports Med*. 2012;40(12):2862-2871.
8. Chung SW, Oh JH, Gong HS, Kim JY, Kim SH. Factors affecting rotator cuff healing after arthroscopic repair: osteoporosis as one of the independent risk factors. *Am J Sports Med*. 2011;39(10):2099-2107.
9. Derwin KA, Baker AR, Iannotti JP, McCarron JA. Preclinical models for translating regenerative medicine therapies for rotator cuff repair. *Tissue Eng Part B Rev*. 2010;16(1):21-30.
10. Deumens R, Jaken RJP, Marcus MAE, Joosten EAJ. The CatWalk gait analysis in assessment of both dynamic and static gait changes after adult rat sciatic nerve resection. *J Neurosci Methods*. 2007;164(1):120-130.
11. Ficklscherer A, Serr M, Loitsch T, et al. The influence of different footprint preparation techniques on tissue regeneration in rotator cuff repair in an animal model. *Arch Med Sci AMS*. 2017;13(2):481-488.
12. Galatz LM, Ball CM, Teefey SA, Middleton WD, Yamaguchi K. The outcome and repair integrity of completely arthroscopically repaired large and massive rotator cuff tears. *J Bone Joint Surg Am*. 2004;86(2):219-224.
13. Gladstone JN, Bishop JY, Lo IKY, Flatow EL. Fatty infiltration and atrophy of the rotator cuff do not improve after rotator cuff repair and correlate with poor functional outcome. *Am J Sports Med*. 2007;35(5):719-728.
14. Gulotta LV, Kovacevic D, Packer JD, Deng XH, Rodeo SA. Bone marrow-derived mesenchymal stem cells transduced with scleraxis

improve rotator cuff healing in a rat model. *Am J Sports Med.* 2011;39(6):1282-1289.

15. Holfeld J, Tepeköylü C, Kozaryn R, et al. Shockwave therapy differentially stimulates endothelial cells: implications on the control of inflammation via toll-like receptor 3. *Inflammation.* 2014;37(1):65-70.

16. Huang T-H, Sun C-K, Chen Y-L, et al. Shock wave enhances angiogenesis through VEGFR2 activation and recycling. *Mol Med (Camb Mass).* 2017;22:850-862.

17. Jiang K, Chun G, Wang Z, Du Q, Wang A, Xiong Y. Effect of transforming growth factor-β3 on the expression of Smad3 and Smad7 in tenocytes. *Mol Med Rep.* 2016;13(4):3567-3573.

18. Kappos EA, Sieber PK, Engels PE, et al. Validity and reliability of the CatWalk system as a static and dynamic gait analysis tool for the assessment of functional nerve recovery in small animal models. *Brain Behav.* 2017;7(7):e00723.

19. Killian ML, Cavinatto L, Shah SA, et al. The effects of chronic unloading and gap formation on tendon-to-bone healing in a rat model of massive rotator cuff tears. *J Orthop Res.* 2014;32(3):439-447.

20. Killian ML, Cavinatto LM, Ward SR, Havlioglu N, Thomopoulos S, Galatz LM. Chronic degeneration leads to poor healing of repaired massive rotator cuff tears in rats. *Am J Sports Med.* 2015;43(10):2401-2410.

21. Kisch T, Wuerfel W, Forstmeier V, et al. Repetitive shock wave therapy improves muscular microcirculation. *J Surg Res.* 2016;201(2):440-445.

22. Leal MF, Astur DC, Debieux P, et al. Identification of suitable reference genes for investigating gene expression in anterior cruciate ligament injury by using reverse transcription-quantitative PCR. *PloS One.* 2015;10(7):e0133323.

23. Lemke A, Penzenstadler C, Ferguson J, et al. A novel experimental rat model of peripheral nerve scarring that reliably mimics post-surgical complications and recurring adhesions. *Dis Model Mech.* 2017;10(8):1015-1025.

24. Maeda Y, Yonemochi Y, Nakajyo Y, Hidaka H, Ikeda T, Ando Y. CXCL12 and osteopontin from bone marrow–derived mesenchymal stromal cells improve muscle regeneration. *Sci Rep.* 2017;7(1):3305.

25. Mariotto S, Cavalieri E, Amelio E, et al. Extracorporeal shock waves: from lithotripsy to anti-inflammatory action by NO production. *Nitric Oxide Biol Chem.* 2005;12(2):89-96.

26. Meirer R, Brunner A, Deibl M, Oehlbauer M, Piza-Katzer H, Kamelger FS. Shock wave therapy reduces necrotic flap zones and induces VEGF expression in animal epigastric skin flap model. *J Reconstr Microsurg.* 2007;23(4):231-236.

27. Melis B, DeFranco MJ, Chuinard C, Walch G. Natural history of fatty infiltration and atrophy of the supraspinatus muscle in rotator cuff tears. *Clin Orthop Relat Res.* 2010;468(6):1498-1505.

28. Mittermayr R, Hartinger J, Antonic V, et al. Extracorporeal shock wave therapy (ESWT) minimizes ischemic tissue necrosis irrespective of application time and promotes tissue revascularization by stimulating angiogenesis. *Ann Surg.* 2011;253(5):1024-1032.

29. Monrad N, Ganestam A, Kallemose T, Barfod KW. Alarming increase in the registration of degenerative rotator cuff-related lesions a nationwide epidemiological study investigating 244,519 patients. *Knee Surg Sports Traumatol Arthrosc.* 2018;26(1):188-194.

30. Newton MD, Davidson AA, Pomajzl R, Seta J, Kurdziel MD, Maerz T. The influence of testing angle on the biomechanical properties of the rat supraspinatus tendon. *J Biomech.* 2016;49(16):4159-4163.

31. Orhan Z, Ozturan K, Guven A, Cam K. The effect of extracorporeal shock waves on a rat model of injury to tendo Achillis: a histological and biomechanical study. *J Bone Joint Surg Br.* 2004;86(4):613-618.

32. Serizawa F, Ito K, Matsubara M, Sato A, Shimokawa H, Satomi S. Extracorporeal shock wave therapy induces therapeutic lymphangiogenesis in a rat model of secondary lymphoedema. *Eur J Vasc Endovasc Surg.* 2011;42(2):254-260.

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(5):383-392.

34. Taniguchi N, Suenaga N, Oizumi N, et al. Bone marrow stimulation at the footprint of arthroscopic surface-holding repair advances cuff repair integrity. *J Shoulder Elbow Surg.* 2015;24(6):860-866.

35. Tepeköylü C, Wang F-S, Kozaryn R, et al. Shock wave treatment induces angiogenesis and mobilizes endogenous CD31/CD34-positive endothelial cells in a hindlimb ischemia model: implications for angiogenesis and vasculogenesis. *J Thorac Cardiovasc Surg.* 2013;146(4):971-978.

36. Tucker JJ, Cirone JM, Morris TR, et al. Pulsed electromagnetic field therapy improves tendon-to-bone healing in a rat rotator cuff repair model. *J Orthop Res.* 2017;35(4):902-909.

37. Valencia Mora M, Antuña Antuña S, García Arranz M, Carrascal MT, Barco R. Application of adipose tissue-derived stem cells in a rat rotator cuff repair model. *Injury.* 2014;45(suppl 4):S22-S27.

38. van der Worp H, van den Akker-Scheek I, van Schie H, Zwerver J. ESWT for tendinopathy: technology and clinical implications. *Knee Surg Sports Traumatol Arthrosc.* 2013;21(6):1451-1458.

39. Walder RY, Wattiez A-S, White SR, Marquez de Prado B, Hamity MV, Hammond DL. Validation of four reference genes for quantitative mRNA expression studies in a rat model of inflammatory injury. *Mol Pain.* 2014;10:55.

40. Wang C-J, Ko J-Y, Chan Y-S, Weng L-H, Hsu S-L. Extracorporeal shockwave for chronic patellar tendinopathy. *Am J Sports Med.* 2007;35(6):972-978.

41. Wang C-J, Wang F-S, Yang KD, et al. Shock wave therapy induces neovascularization at the tendon-bone junction: a study in rabbits. *J Orthop Res.* 2003;21(6):984-989.

42. Weihs AM, Fuchs C, Teuschl AH, et al. Shock wave treatment enhances cell proliferation and improves wound healing by ATP release-coupled extracellular signal-regulated kinase (ERK) activation. *J Biol Chem.* 2014;289(39):27090-27104.

43. Yamaguchi T, Ochiai N, Sasaki Y, et al. Efficacy of hyaluronic acid or steroid injections for the treatment of a rat model of rotator cuff injury. *J Orthop Res.* 2015;33(12):1861-1867.

44. Yamamoto A, Takagishi K, Osawa T, et al. Prevalence and risk factors of a rotator cuff tear in the general population. *J Shoulder Elbow Surg.* 2010;19(1):116-120.

45. Yamazaki H, Ochiai N, Kenmoku T, et al. Assessment of pain-related behavior and pro-inflammatory cytokine levels in the rat rotator cuff tear model. *J Orthop Res.* 2014;32(2):286-290.

46. Yan X, Zeng B, Chai Y, Luo C, Li X. Improvement of blood flow, expression of nitric oxide, and vascular endothelial growth factor by low-energy shockwave therapy in random-pattern skin flap model. *Ann Plast Surg.* 2008;61(6):646-653.