

Hyperbaric oxygen treatment promotes tendon–bone interface healing in a rabbit model of rotator cuff tears

Hongqiu Li^{1, #}, Meiling Xiao^{2, #}, Feng Yang³, Zhonghai Zhao², Liang A^{1, *}

<https://doi.org/10.4103/mgr.MEDGASRES-D-24-00034>

Date of submission: May 14, 2024

Date of decision: May 31, 2024

Date of acceptance: July 4, 2024

Date of web publication: August 31, 2024

Abstract

Due to the high-intensity pressure that the shoulder cuff endures, it is prone to traumas and tears. The main critical function of the shoulder cuff muscles is to effectively facilitate shoulder movement and securely maintain the humeral head in the precise center of the joint cavity to prevent superior migration during abduction processes. Shoulder cuff injuries typically involve the muscle–tendon–bone interface, but existing repair techniques do not always guarantee complete and secure healing, leading to retears. Hyperbaric oxygen therapy, as an auxiliary treatment, can significantly promote the muscle–tendon–bone healing process. To explore the impact of hyperbaric oxygen therapy on the bone–tendon interface healing process in a rabbit model specifically designed for shoulder cuff tears, an experiment was conducted on New Zealand white rabbits by performing a full-thickness tear of the supraspinatus tendon in the left shoulder, followed by 2 hours per day of 100% oxygen treatment at 2 absolute atmospheres for 5 days. The results indicate that hyperbaric oxygen therapy significantly enhances vascularization at the interface between the shoulder cuff and tendon–bone, promotes collagen fiber regeneration in the tendon, improves the tensile strength of the tendon–bone complex, and does not have a significant effect on biomechanical stability. This suggests that hyperbaric oxygen therapy has a significant positive impact on the histological and biomechanical healing of shoulder cuff tears in rabbits, expediting the healing process of the tendon–bone interface.

Key Words: biomechanics; collagen; fibers; bone interface; histological change; vascularization; inflammation; rotator cuff tissue engineering; adjunct therapy

Introduction

The rotator cuff, which is known to be the most powerful and thickest tendon in the human body, is especially likely to suffer from trauma and tears because it is subjected to high levels of stress.¹ The primary and crucial function of the rotator cuff muscles is to effectively facilitate the movement of the shoulder and to firmly maintain the humeral head centered precisely within the glenoid cavity to prevent superior translation during abduction.² Consequently, a tear in the rotator cuff will have a significant impact on the functionality of the shoulder during activities carried out in daily life. As society progresses toward an aging stage, shoulder cuff disorders are being increasingly recognized as a prominent public health concern. Rotator cuff injuries often involve the tendon–bone interface, which brings about great challenges for achieving effective healing.³ Rotator cuff tendon–bone healing involves complex transitional zones such as the tendon. Fibrocartilage, along with mineralized fibrocartilage and bone, poses a continual and significant challenge for orthopedic and sports medicine practitioners worldwide.⁴ Recent in-depth research carried out in various disciplines, such as biomaterials, cell biology, and biomechanics, has focused on restoring the structure of this interface, which is highly important for restoring shoulder joint function and

preventing retears. However, the existing repair techniques do not always guarantee thorough and completely secure healing, which then leads to the recurrence of tears.

Hyperbaric oxygen treatment (HBOT) has progressively emerged as a possible additional treatment approach to prominently enhance the tendon–bone healing process. Through the delivery of oxygen under high pressure, HBOT has the potential to enhance tissue oxygenation, boost angiogenesis, and regulate inflammatory responses, thereby promoting tissue repair processes.^{5,6} HBOT significantly boosts tissue oxygenation, which is crucial for cellular metabolism and collagen synthesis, and promotes angiogenesis to strengthen the blood supply and nutrient delivery, which are indispensably necessary for tissue repair.⁷ Furthermore, the anti-inflammatory effects of HBOT can significantly reduce the production of proinflammatory cytokines⁸ and thereby establish a conducive atmosphere for the process of healing. Furthermore, HBOT can remarkably stimulate the proliferation of fibroblasts, the deposition of collagen, and the process of tissue remodeling,⁹ strengthening the tendon–bone interface.¹⁰

Our in-depth study attempts to comprehensively explore the various effects of HBOT on tendon–bone complex healing in a rabbit model of rotator cuff tears, concentrating not only on its

¹Department of Orthopedics, Central Hospital Affiliated to Shenyang Medical College, Shenyang, Liaoning Province, China; ²Department of Rehabilitation, Central Hospital Affiliated to Shenyang Medical College, Shenyang, Liaoning Province, China; ³Department of Medical Imaging, Central Hospital Affiliated to Shenyang Medical College, Shenyang, Liaoning Province, China

*Correspondence to: Liang A, MD, aliang36@163.com.

<https://orcid.org/0000-0002-6505-550X> (Hongqiu Li)

#Both authors contributed equally to this work.

How to cite this article: Li H, Xiao M, Yang F, Zhao Z, A L. Hyperbaric oxygen treatment promotes tendon–bone interface healing in a rabbit model of rotator cuff tears. *Med Gas Res.* 2025;15(1):164-170.

influence during the early periods of tendon healing but also on thoroughly elucidating the specific mechanisms involved in promoting neovascularization and optimizing the quality of collagen fibers. This research offers a novel perspective for understanding the mechanisms of HBOT in the treatment of shoulder cuff injuries, laying a solid foundation for the future development of individualized therapeutic strategies.

Materials and Methods

The protocol was successfully approved by the Animal Ethics Committee of Central Hospital Affiliated to Shenyang Medical College (approval No. 2023038). Premedication via intravenous injection of Zoletil (medetomidine (7 mg/kg; Virbac, Carros, Provence-Alpes-Côte d'Azur, France) and xylazine (2 mg/kg; Bayer, Leverkusen, North Rhine-Westphalia, Germany) was administered to minimize pain, distress, and potential death in the animals as much as possible.

This study utilized 32 male New Zealand White rabbits acquired from the Animal Laboratories of Shenyang Medical College (license No. SCXK (Liao) 2020-0001), and these rabbits had an average age of 16 weeks. The ages of the animals were evenly distributed among all the groups, and the average weight ranged from 2.5 to 3.5 kg. All rabbits were placed and housed in standard rabbit cages and acclimatized for 4 weeks under a 12-hour light–dark cycle within a room temperature range of $22 \pm 3^\circ\text{C}$ and a humidity of 45%. The rats were given unrestricted access to food and municipal tap water freely provided. Before the surgery, the rabbits fasted for 12 hours.

All rabbits underwent an identical repair process involving the establishment of a full-thickness rotator cuff tear model by means of rabbit infraspinatus and supraspinatus muscle tendons on the left shoulder (**Figure 1**), while on the right shoulder, a sham surgery was carried out where only the tendon was exposed without generating a tear. The rabbits were sorted in sequence from the lightest to the heaviest according to their weights and subsequently were randomly separated into two groups, namely, the model group and the HBOT group, with sixteen rabbits in each group. The randomization procedure was carried out by means of a random number approach in accordance with the designated numbers.

The HBOT group was exposed daily to 100% oxygen at 2 absolute atmosphere (ATA; 1 ATA = 101 kPa) for 2 hours continuously over 5 successive days, while the model group

was subjected to normal air.

Histological assessment

Detailed histological evaluations were carried out by an experienced pathologist who was deliberately kept unaware of the study protocol and the group assignments. Four rabbits were euthanized at 4, 8, and 12 weeks after injury.

The entire humerus, along with the infraspinatus and supraspinatus tendon as well as the muscle, was painstakingly and meticulously harvested. The specimens were firmly fixed in neutral buffered 10% formalin (Sbjbio, Nanjing, China) for a period of 2 days and then decalcified with 20% formic acid for 2 weeks, followed by dehydration through graded alcohol and clearing in xylene. Before performing the histochemical analysis, all the specimens were subjected to a macroscopic assessment to evaluate the extent of healing (whether it was absent, partial, or complete). Decalcification was then performed. Subsequently, the specimens were embedded in paraffin wax, and 5-mm long longitudinal sections were cut parallel to the long axis of both the infraspinatus and supraspinatus tendons, encompassing the proximal humerus, the infraspinatus and supraspinatus tendons, and the muscle. Successive serial sections were cut to a thickness of precisely 3 mm and then stained with hematoxylin and eosin (Sigma-Aldrich, Shanghai, China) for the purpose of evaluating the overall general morphology.

The samples were evaluated for tendon-to-bone healing by means of a light microscope (BX51; Olympus, Tokyo, Japan) through a pathologist who was blinded to the specific treatments. Photomicrographs were precisely captured through the use of a digital camera (DP72; Olympus). The scoring system was specifically adapted from histological evaluation scores.^{11,12} Seven parameters, namely, fiber structure, fiber arrangement, the rounding state of the nuclei, inflammation, enhanced vascularity, cell density, and the stainability of collagen, were evaluated by means of a semiquantitative histological scoring system. These various variables were graded within a scale ranging from 0 to 3, where 0 represents a state of normalcy and 3 represents the highest level of abnormality. Consequently, a completely normal tendon was assigned a score of 0, while a tendon that was severely abnormal was assigned a score of 21 (**Table 1**). Three sections were randomly selected from each sample and then blindly evaluated by three independent assessors in a row to obtain an average score for comparison.

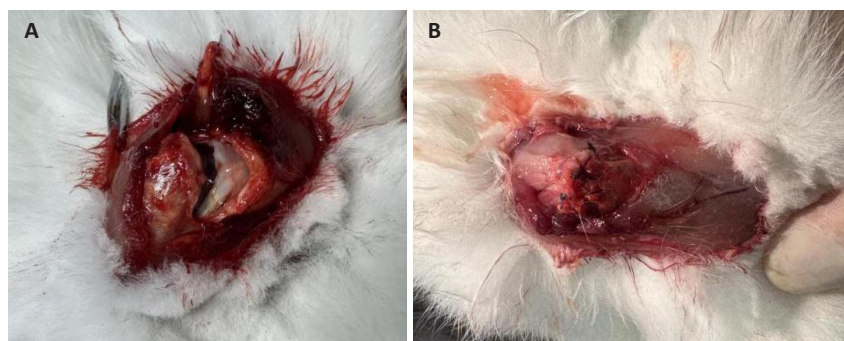


Figure 1 | Surgical procedure for the rotator cuff tear model in rabbits under operation field view.

(A) An animal model with rotator cuff tears. (B) A rabbit after rotator cuff repair surgery.

Table 1 | Scoring system for histological results

	Tendon pathologic score			
	0	1	2	3
Fiber structure	Continuous, long fiber	Slightly fragmented	Moderately fragmented	Severely fragmented
Fiber arrangement	Compacted, parallel	Slightly rounded	Moderately rounded	Severely rounded
Rounding of nuclei	Long spindle shape	Slightly rounded	Moderately rounded	Severely rounded
Inflammation (area infiltrated by inflammatory cells) (%)	< 10	10–20	20–30	> 30
Increased vascularity (%)	< 10	10–20	20–30	> 30
Cell density	Normal pattern	Slightly increased	Moderately increased	Severely increased
Collagen stainability	Normal staining	Slightly reduced	Moderately reduced	Severely reduced

Biomechanical evaluation

Biomechanical evaluations were carried out by an experienced biomechanical specialist who had no knowledge of the study protocol or group allocations. At 12 weeks after the operation, 4 rabbits in each group died.

A specifically designed custom fixture clamping system was used to test the rabbit tendon–bone complex. After euthanasia, the entire rotator cuff of both shoulders, together with the humerus of each rabbit, was removed 12 weeks after surgery. Then, the specimens were placed and fixed in the load frame for uniaxial tensile loading, precisely aligning with the direction of the pull of the tendon–bone complex. The proximal portion of the humerus was firmly secured by means of clamps, and the tendon was firmly gripped in a special custom-made gripping device with serrated edges to ensure the stable holding of the soft tissue and effectively prevent any slippage.

Preconditioning of the tendon–bone complex and the humeral head was carried out with a static preload of precisely 0 N for 5 minutes before the tensile test. Immediately after preconditioning, the ultimate load leading to failure was then precisely noted under uniaxial tension at a rate of 30 mm/min. Failure was ascertained by a sudden decline in the load–displacement curve, the appearance of a fracture in the bony constituents, or a total rupture in the soft tissue elements.

The primary outcome parameter in the biomechanical tests was the ultimate failure load (N), which specifically measured the pull-out strength of the healed rotator cuff, along with the yield force and the vertical displacements at the time of failure. The elongation at failure was specifically defined as the assessment of the vertical displacement of the movement arm of the test machine when it reached the point of failure. The mechanical properties of the normal rotator cuff on the side that underwent sham surgery were evaluated in the same way in a comprehensive manner.

Statistical analysis

We used the Wilcoxon test to compare the tensile strength between the sham and the other side in the case of nonparametric variables, and the Mann–Whitney *U* test using SPSS 22 (IBM Corp., Armonk, NY, USA) was used for nonparametric variables to compare the semiquantitative

histological evaluation scores and the tensile strength.

Results

At the time of harvest, there were no obvious gaps between the tendon and bone interfaces in any of the specimens.

Histological assessment

To accurately determine the position suitable for histological analysis, the sections were initially inspected under relatively low magnification. The tendinous part of the tendon–bone complex precisely at the interface between the tendon and the bone was selected. Then, the histological evaluation scores of the tendon were evaluated by utilizing higher magnification photomicrographs in a detailed manner.

At 4 weeks, both groups showed hypercellularity and hypervascularity precisely at the implant location. The majority of the tendon–bone complex was basically covered with a considerable amount of fibrous tissue as well as inflammatory cells. Collagen fibers showed rather poor organization, and they formed a moderately wavy configuration. It was obvious that there was clear fiber fragmentation, and the tenocyte nuclei presented a moderately rounded appearance. There were no notable differences between the two groups). At 8 weeks, the vascularity significantly decreased in both groups, and inflammatory cellular infiltration was minimized to a considerable extent. The quantity of collagen fibers distinctly increased in all of the samples. However, the HBOT group presented a more compact and denser fiber arrangement than did the model group. At 12 weeks, the tenocyte nuclei seemed to be flattened, had a spindle-shaped appearance, and were sometimes even arranged in orderly rows. Notably, the HBOT group presented a more densely packed and neatly parallel collagen fiber structure. In contrast, the model group presented a relatively less fibrillary pattern than the HBOT group (**Figure 2**).

At 8 weeks, the histological scores of the model group in terms of fiber structure, angiogenesis, and collagen stainability were significantly lower than those of the HBOT group. The cell density and degree of inflammation presented similar tendencies in both groups at 4, 8, and 12 weeks. At 8 and 12 weeks, the histological scores of the model group were significantly lower than those of the HBOT group ($P < 0.05$; **Figure 3**).

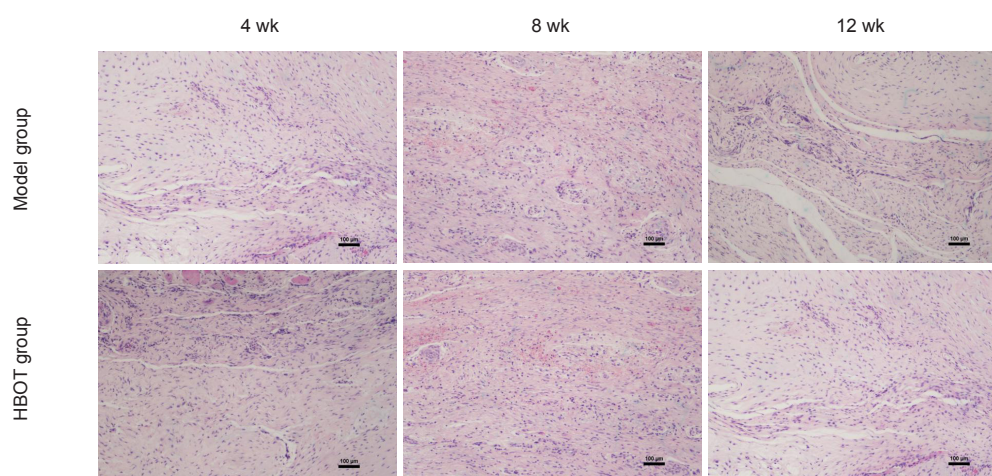


Figure 2 | Effect of HBOT on the histological changes in the tendon–bone interface in a rabbit model of rotator cuff tear, as determined by hematoxylin and eosin staining.

At 4 weeks, at the tendon–bone interface, the tendinous portion of the supraspinatus was selected. In both groups, most of the supraspinatus tendons were covered with fibrous tissue and inflammatory cells. The collagen fibers were poorly organized and moderately wavy. Fiber fragmentation was clearly visible, and the tenocyte nuclei were moderately rounded. At 8 weeks, there was reduced infiltration of inflammatory cells in both groups. The number of collagen fibers increased in all the samples. However, the experimental group exhibited a denser arrangement of fibers than did the model group. At 12 weeks, the tendon cell nuclei appeared flattened, spindle-shaped, and sometimes arranged in rows. However, the HBOT group exhibited more compact and parallel collagen fibers. In contrast, the model group showed a less fibrillar pattern than did the experimental group. Scale bars: 100 μ m. HBOT: Hyperbaric oxygen treatment.

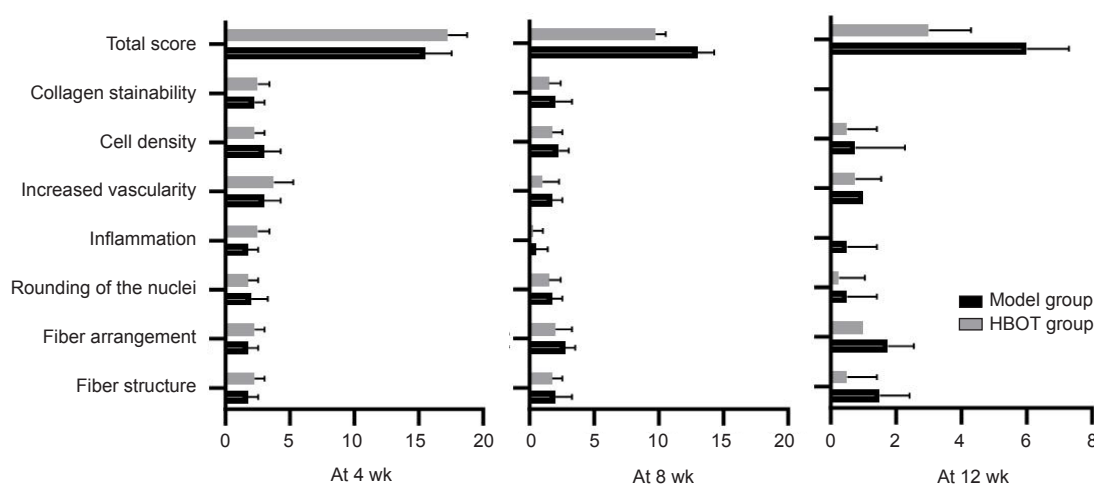


Figure 3 | Histological evaluation scores of supraspinatus tendon repair.

Data are expressed as the mean $\pm$ SD ($n = 4$). * $P < 0.05$ (Mann–Whitney U test). HBOT: Hyperbaric oxygen treatment.

Biomechanical results

After 12 weeks, the tensile strength of the tissue samples was assessed. The mean failure load of the HBO group differed markedly between the operated side and the sham surgery side. Similarly, the failure load on the operated side in the model group was obviously and significantly lower than that on the operated side in the sham surgery group. Moreover, the load to failure on the operated side within the HBOT group was noticeably greater than that in the model group. These findings were in line with the experimental results related to yield force and vertical displacement.

Remarkable differences between the two groups were observed in terms of the yield force at the 12th postoperative

week. The maximum forces that both the model group and the HBOT group were capable of withstanding were 39.50 ± 2.08 N and 51.00 ± 2.94 N, respectively ($P < 0.05$; **Figure 4A**). On average, the maximum force within the HBOT group was distinctly and significantly greater than that within the model group. Similarly, in the HBOT group, there was a significant difference in the vertical displacement. The average vertical displacements of the model group and the HBOT group were 29.5 ± 3.11 and 37.75 ± 1.71 N/mm, respectively ($P < 0.05$; **Figure 4B**).

All the failures occurred at the tendinous part of the tendon–bone complex rather than precisely at the interface between the tendon and the bone.

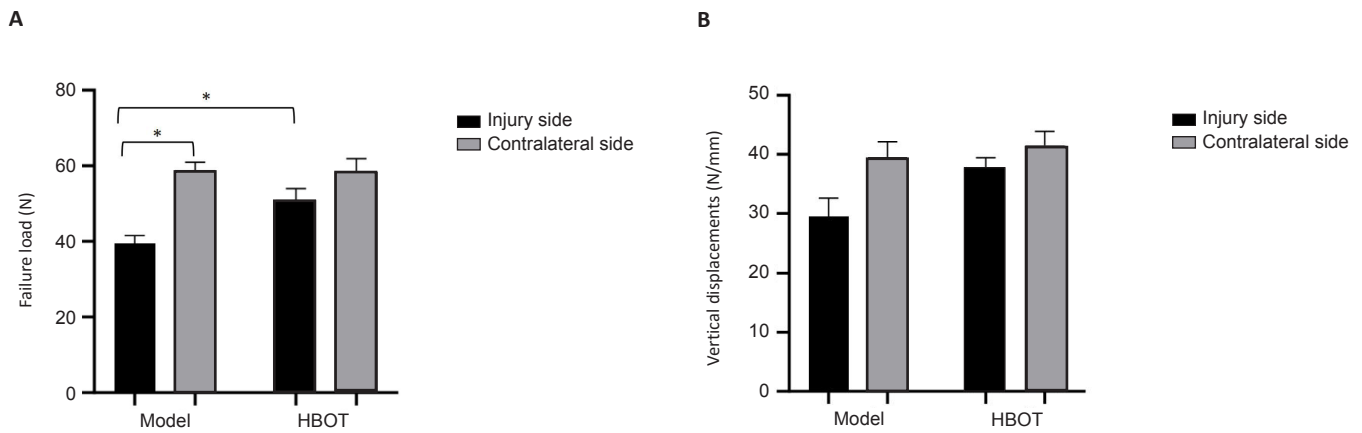


Figure 4 | Biomechanical assessment of the ultimate failure load (A) and elongation at failure (B) between the HBOT and model groups at 12 weeks.

Data are expressed as the mean $\pm$ SD ($n = 4$). * $P < 0.05$ (Mann–Whitney U test). HBOT: Hyperbaric oxygen treatment.

The differences that were detected among the groups with respect to biomechanical parameters were in line with the findings of Mashitori et al.¹³ in a consistent manner. Additionally, Horn et al.¹⁴ demonstrated that the application of HBOT has a significant impact on the biomechanical healing process of the medial collateral tendon by the 12th week, showing marked effects. In this particular study, the 12th postoperative week provided ample time for the rotator cuff of the rabbits to heal to a state of biomechanical proficiency.

Discussion

With remarkable progress made in the surgical techniques and equipment utilized for mending rotator cuff tears, the postoperative clinical results have significantly improved. However, there are still challenges that exist, including a rather high rate of unsuccessful repairs.¹⁵ Sporting injuries often require treatment for 3 to 10 sessions. Recently, HBOT for sports-related musculoskeletal injuries has attracted increased amounts of attention.¹⁶ HBOT has increasingly gained wide popularity among injured athletes because of its supposed advantages in promoting the speed of recovery, especially among professional athletes or those who possess considerable financial resources. Despite its extensive popularity, the evidence supporting the effectiveness of HBOT in this particular context remains rather limited. Our research selected rabbits because they are commonly utilized in orthopedic studies and have obvious advantages in establishing models of shoulder cuff injuries. Compared with previous studies on the shoulder structure of rabbits, the anatomical structure of the rabbit scapula and the path of the shoulder cuff tendons are relatively more primitive. Compared with the shoulder cuff structure in humans, the anatomical structure of the rabbit scapula is relatively more primitive in nature.¹⁷ Furthermore, rabbits are moderate in size, are cost-efficient, are rather easy to raise, and thereby offer inherent advantages in regard to conducting animal experiments.

HBOT specifically involves the administration of 100% oxygen

within a pressure vessel where the pressure is set at a level higher than that of the normal atmospheric pressure. Various HBOT models have been investigated in the literature. Ishii et al.¹⁸ indicated that a pressure of 2 ATA for 60 minutes plays a more significant role in promoting the formation of fibers and ligaments than other pressures. Mashitori et al.¹⁶ applied HBOT for 2 hours at 2 ATA in their ligament healing experiment. Similarly, Yeh et al.¹⁹ used HBOT for 2 hours at 2 ATA in a rabbit experimental study. In our study, we applied HBOT at 2 ATA for 2 hours to fully utilize the optimal impacts of oxygen on the process of wound healing. Zhao et al.²⁰ firmly established a direct and significant correlation between oxygen and growth factors. Consequently, it is rational that HBOT promotes the ingrowth of blood vessel formation, which is related to the enhancement of blood supply, leading to an increase in trabecular bone around the tendon as well as an improvement in the contact between the tendon and the bone at the tendon–bone interface. A number of different theories have been proposed in relation to the cause of rotator cuff injuries. The chronic degeneration theory suggests that tendon degeneration is capable of resulting in rupture regardless of the degree of tendon overload,²¹ and this is what it postulates. Among the suspected susceptibility factors are recurrent microtrauma, the process of aging, and localized hypovascularity.²² Angiographic and histological findings firmly support the degeneration theory, clearly revealing scattered regions of healing and mucoid as well as edematous alterations in the tissue samples, which are strongly indicative of chronic progression. Tissue oxygenation is widely known to be of great significance for the process of healing, and HBOT is considered to be able to boost wound healing by increasing the levels of dissolved oxygen in blood and tissues.²³ Furthermore, HBOT contributes to the process of neovascularization in tissues with relatively reduced vascularity.^{7,24} Research has demonstrated a direct connection between oxygen and growth factors, indicating that HBOT promotes the growth of blood vessels, which leads to the improvement of blood supply and the reinforcement

of trabecular bone formation around the tendon, thereby enhancing contact at the tendon–bone interface.^{5,16,25}

The exact mechanism by which HBOT promotes healing remains unknown. In previous studies, the potential mechanisms and physiological effects of HBOT were clearly described. In a relatively short period, HBOT can enhance the delivery of oxygen through vasoconstriction, effectively reducing edema, significantly improving the phagocytic function of neutrophils, exerting remarkable anti-inflammatory effects, and alleviating ischemia–reperfusion injury.²⁶ With repeated administration carried out over longer durations, HBOT can induce neovascularization, neoangiogenesis, and collagen generation, which has the potential to significantly enhance muscle rehabilitation during the inflammatory and proliferative stages of the recovery process.²⁷ Moreover, further studies have shown that HBOT can induce oxidative stress by controlling the generation of reactive oxygen and nitrogen species, thereby activating various cellular processes and pathways.^{7,28} Key mechanisms include increased growth factor (e.g., hypoxia-inducible factor 1 α), vascular endothelial growth factor, stromal-derived factor 1, the mobilization of bone marrow-derived stem cells (CD34⁺ cells), and the reduction of neutrophil adhesion (by means of the modification of integrin β 2), which can alleviate ischemia–reperfusion injury.²⁹

Our in-depth study revealed that there was no marked difference in biomechanical resistance among the groups that were treated with HBOT after refixation and those that underwent sham surgery. In other words, the stability after 12 weeks was comparable to the stability without tensile tearing forces, which can actually be ascribed to HBOT. Based on the obtained experimental results, HBOT has emerged as an important treatment modality that has the potential to significantly improve therapeutic outcomes in patients with rotator cuff tears. Consequently, our histological findings imply that the group treated with HBOT entered the remodeling stage ahead of time and exhibited a more mature healing pattern. Biomechanical analyses more thoroughly demonstrated the outstanding superiority of HBOT treatment compared to cuff repair alone. All the cases of failure were distinctly observed precisely at the tendinous part of the supraspinatus rather than at the interface between the bone and the tendon. This clearly shows that tendon–to–bone healing was successfully accomplished in all the specimens by the 12-week postoperative period.

Our study has several limitations. First, the specific mechanism or detailed mechanism by which HBOT improves the healing of acute rotator cuff tears is still not fully understood. Second, the nonexistence of a chronic tear model restricts our capacity to extend these discoveries to chronic cuff tears because different mechanisms or cellular settings may be implicated in acute as opposed to chronic injuries. Furthermore, the animal model employed might not precisely simulate the degenerative age-related rotator cuff tears that are typically observed in humans, in which intrinsic degenerative alterations in tendon retraction are rather

widespread. Further investigations are needed to validate these hypotheses. However, this study has several strengths. Only a very small number of previous studies have specifically carried out investigations and in-depth analyses on the effects of HBOT, particularly for rotator cuff repair. Furthermore, in this animal study, histological changes were evaluated at different postoperative time intervals in a meticulous manner. The outcomes of the semiquantitative assessment clearly revealed marked differences between the HBOT and model groups, strongly highlighting the effectiveness of HBOT in facilitating distinct histological alterations that are associated with enhanced healing procedures.

In conclusion, although the present study ultimately offers a very valuable understanding of the potential of HBOT as a new therapeutic means for promoting the healing of rotator cuffs, more in-depth research is still required to confirm its clinical effectiveness, optimize treatment plans, and clarify the underlying mechanisms involved. Collaborative endeavors among clinicians, researchers, and industry partners are vital for promoting our understanding of HBOT in the management of rotator cuff tears and converting these discoveries into enhanced patient care.

Author contributions: HL was assigned responsibility for conducting the literature review, preparing the animal model, sectioning, and documenting the experimental procedures. MX meticulously collected the experimental data, conducted the in-depth statistical analysis, and then drafted the manuscript in detail. FY collected the experimental data and information. ZZ was responsible for conducting the mechanical experiments and meticulously recorded the detailed experimental procedures. LA was responsible for the design of the experiment and the supervision of the entire experimental procedure.

Conflicts of interest: The authors explicitly state and declare that they have no potential or existing conflicts of interest.

Data availability statement: All relevant data are within the paper.

Open access statement: This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

References

1. Huegel J, Williams AA, Soslowky LJ. Rotator cuff biology and biomechanics: a review of normal and pathological conditions. *Curr Rheumatol Rep*. 2015;17:476.
2. Hess SA. Functional stability of the glenohumeral joint. *Man Ther*. 2000;5:63-71.
3. Suh DS, Lee JK, Yoo JC, et al. Atelocollagen enhances the healing of rotator cuff tendon in rabbit model. *Am J Sports Med*. 2017;45:2019-2027.
4. Wang HN, Rong X, Yang LM, Hua WZ, Ni GX. Advances in stem cell therapies for rotator cuff injuries. *Front Bioeng Biotechnol*. 2022;10:866195.
5. Gupta M, Rathored J. Hyperbaric oxygen therapy: future prospects in regenerative therapy and anti-aging. *Front Aging*. 2024;5:1368982.

6. Re K, Gandhi J, Liang R, et al. Clinical utility of ozone therapy and hyperbaric oxygen therapy in degenerative disc disease. *Med Gas Res.* 2023;13:1-6.
7. De Wolde SD, Hulskes RH, Weenink RP, Hollmann MW, Van Hulst RA. The effects of hyperbaric oxygenation on oxidative stress, inflammation and angiogenesis. *Biomolecules.* 2021;11:1210.
8. de Wolde SD, Hulskes RH, de Jonge SW, et al. The effect of hyperbaric oxygen therapy on markers of oxidative stress and the immune response in healthy volunteers. *Front Physiol.* 2022;13:826163.
9. Gould LJ, May T. The science of hyperbaric oxygen for flaps and grafts. *Surg Technol Int.* 2016;28:65-72.
10. Leite CBG, Leite MS, Varone BB, et al. Hyperbaric oxygen therapy enhances graft healing and mechanical properties after anterior cruciate ligament reconstruction: an experimental study in rabbits. *J Orthop Res.* 2024;42:1210-1222.
11. Chen J, Yu Q, Wu B, et al. Autologous tenocyte therapy for experimental Achilles tendinopathy in a rabbit model. *Tissue Eng Part A.* 2011;17:2037-2048.
12. Maffulli N, Ewen SW, Waterston SW, Reaper J, Barrass V. Tenocytes from ruptured and tendinopathic achilles tendons produce greater quantities of type III collagen than tenocytes from normal achilles tendons. An in vitro model of human tendon healing. *Am J Sports Med.* 2000;28:499-505.
13. Mashitori H, Sakai H, Koibuchi N, et al. Effect of hyperbaric oxygen on the ligament healing process in rats. *Clin Orthop Relat Res.* 2004;268-274.
14. Horn PC, Webster DA, Amin HM, Mascia MF, Werner FW, Fortino MD. The effect of hyperbaric oxygen on medial collateral ligament healing in a rat model. *Clin Orthop Relat Res.* 1999:238-242.
15. Longo UG, Carnevale A, Piergentili I, et al. Retear rates after rotator cuff surgery: a systematic review and meta-analysis. *BMC Musculoskelet Disord.* 2021;22:749.
16. Moghadam N, Hieda M, Ramey L, Levine BD, Guilliod R. Hyperbaric oxygen therapy in sports musculoskeletal injuries. *Med Sci Sports Exerc.* 2020;52:1420-1426.
17. Quigley RJ, Gupta A, Oh JH, et al. Biomechanical comparison of single-row, double-row, and transosseous-equivalent repair techniques after healing in an animal rotator cuff tear model. *J Orthop Res.* 2013;31:1254-1260.
18. Ishii Y, Ushida T, Tateishi T, Shimojo H, Miyana Y. Effects of different exposures of hyperbaric oxygen on ligament healing in rats. *J Orthop Res.* 2002;20:353-356.
19. Yeh WL, Lin SS, Yuan LJ, Lee KF, Lee MY, Ueng SW. Effects of hyperbaric oxygen treatment on tendon graft and tendon–bone integration in bone tunnel: biochemical and histological analysis in rabbits. *J Orthop Res.* 2007;25:636-645.
20. Zhao LL, Davidson JD, Wee SC, Roth SI, Mustoe TA. Effect of hyperbaric oxygen and growth factors on rabbit ear ischemic ulcers. *Arch Surg.* 1994;129:1043-1049.
21. Osborne JD, Gowda AL, Wiater B, Wiater JM. Rotator cuff rehabilitation: current theories and practice. *Phys Sportsmed.* 2016;44:85-92.
22. Tashjian RZ. Epidemiology, natural history, and indications for treatment of rotator cuff tears. *Clin Sports Med.* 2012;31:589-604.
23. Fosen KM, Thom SR. Hyperbaric oxygen, vasculogenic stem cells, and wound healing. *Antioxid Redox Signal.* 2014;21:1634-1647.
24. Guan Y, Niu H, Liu Z, et al. Sustained oxygenation accelerates diabetic wound healing by promoting epithelialization and angiogenesis and decreasing inflammation. *Sci Adv.* 2021;7:eabj0153.
25. Hsieh CP, Chiou YL, Lin CY. Hyperbaric oxygen-stimulated proliferation and growth of osteoblasts may be mediated through the FGF-2/MEK/ERK 1/2/NF-κB and PKC/JNK pathways. *Connect Tissue Res.* 2010;51:497-509.
26. Kurbel S, Ćurković V, Kovačić B. Hypothesis: Drainage of the peripheral tissue edema by the hyperbaric oxygen therapy because of hyperoxygenation that constricts arterioles and alters the downstream capillary fluid traffic in affected tissues. *Bioessays.* 2023;45:e2300023.
27. Lifson N, Salloum G, Kurochkin P, Bivona M, Yin HY, Alpert S. Treatment outcomes on neovascularization after CRAO treated with hyperbaric oxygen. *Undersea Hyperb Med.* 2021;48:425-430.
28. Schottlender N, Gottfried I, Ashery U. Hyperbaric oxygen treatment: effects on mitochondrial function and oxidative stress. *Biomolecules.* 2021;11:1827.
29. Milovanova TN, Bhopale VM, Sorokina EM, et al. Hyperbaric oxygen stimulates vasculogenic stem cell growth and differentiation in vivo. *J Appl Physiol (1985).* 2009;106:711-728.