Efficacy and Safety of Hyperbaric Oxygen Therapy in Ligament and Tendon Injuries: A Systematic Review

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ABSTRACT

Background

Ligament and tendon injuries are one of the major health concerns that affect over 1.71 billion people around the world. They cause functional limitations and affect the quality of life of people. As conventional methods have their limitations, hyperbaric oxygen therapy (HBOT) is becoming a potential solution for the improvement and acceleration of the healing process in ligament and tendon injuries.

Objective

This systematic review aims to evaluate efficacy and safety of hyperbaric oxygen therapy (HBOT) for ligament and tendon injuries**.**

Methods

This systematic review provides a comprehensive analysis by following PRISMA guidelines. We looked for articles published between March 1999 and May 2024 across 6 databases. The articles included investigated the use of hyperbaric oxygen therapy to treat ligament or tendon injuries. Animal studies, as well as human studies, were included in this review. Studies were evaluated for HBOT, and if they were not related or with insufficient data, they were excluded. Risk of Bias has been assessed using the ROBINS-I tool.

Results

A total of 13 studies were included in the review, with 693 participants. This study has analyzed the effectiveness of HBOT in two ways, namely, standalone treatment and combined methods like HBOT and other methods like platelet growth factor, steroid injections, intermittent oxygen therapy, or platelet-rich plasma. The pressure observed in this study is between 1.3 to 2.8 atmospheres absolute. Compared to the control, the HBOT method improved the repair of tendon or ligament injuries in the participants. The improvements in the participants have been measured using various methods, and the variables used in this method were functional outcomes, biomechanical outcomes, radiological outcomes, histological outcomes, and biochemical outcomes. The studies show that HBOT has increased collagen density, fiber alignment, and

synthesis. Combination of HBOT with other methods has shown a good effect in the tendon and ligament repair. As the ROBINS-I has shown low risk of bias, this makes the study findings reliable.

Conclusion

HBOT seems to be a safe and effective method for speeding up the healing process of tendons and ligaments. But, there is a need for more studies with more number of population for analysing the effect of HBOT in a long run. It is necessary to make a standard protocol for the HBOT treatment method.

Keywords: Efficacy, Hyperbaric oxygen therapy, HBOT, ligament and tendon injuries.

INTRODUCTION

Musculoskeletal problems and Injuries in tendons and ligaments as they affect the normal functioning and day-to-day life of an individual. Over 1.71 billion people are suffering from musculoskeletal issues globally [1]. More than 50 percent of muscle and skeletal problems are related to injuries to ligaments and tendons [2]. Every year, there are over 17 million people affected by ligament injuries, and they need medical treatment in the United States. The estimated cost for the medical treatment is over 40 billion dollars [3]. Along with the economic cost, these injuries significantly affect the patient's quality of life and their ability in occupation, recreation, and health goals. In most cases, the tendons and ligament injuries are usually sprains and strains. They heal without any surgical intervention. However, the process is very slow, and inferior scar tissues may be formed. These tissues may take years to change into a functional tissue [4].

There are several methods used for the treatment of ligament injuries as well as tendon injuries. Some of the methods are surgery, tissue engineering, and other methods. However, these methods sometimes fail to provide a full restoration of the function on the injured site. they also take more time to heal and do not provide an optimal result [5]. Other methods, like platelet-rich plasma, platelet growth factor-bb, and mesenchymal stromal cells, have shown some promise in enhancing the process of healing, but their clinical efficacy has not yet been identified. This is mainly due to the inconsistency in reporting and techniques used in preparation [6]. Another technique using Hyperbaric Oxygenation, HBOT, is used to treat ligament and tendon injuries. This method has provided an enhanced acceleration in the healing process compared to the controls.

This HBOT method is safe, non-invasive, and effective and can treat various conditions. In HBOT, 100% oxygen is administered under increased atmospheric pressure, which results in the dissolving of an increased amount of oxygen in plasma. This increases the oxygen diffusion to the tissue, thus ensuring that necessary oxygen levels reach the injured site. This method has been reported to positively affect cell and tissue recovery acceleration [7][8].

This systematic review mainly aims to assess the safety and efficacy of HBOT for ligament or tendon injuries from the literature. It also provides the study design used, interventions involved in each study, and outcomes obtained for the treatment of tendon and ligament injuries with HBOT.

In addition, the risk of bias helped identify any bias in the research and treatment methods used in the study.

METHODS

This systematic review compiles the statement of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) to analyze the data collection and the outcome [9].

Search strategy

A complete search was made using six electronic databases: PubMed, Embase, Science Direct, Google Scholar, clinicaltrials.gov, and Cochrane Library. The search covered the English language between March 1999 and May 2024. The search terms used are ((((Hyperbaric Oxygen Therapy) AND (Ligament)) OR (Tendon Injuries)) AND (Efficacy)) AND (Safety), (Animal Models) OR (Animal Studies)) OR (Alternative therapies)) AND (Hyperbaric Oxygen Therapy) AND (Ligament)) OR (Tendon Injuries)) AND (Efficacy)) AND (Safety) OR ((HBOT) OR (HBOT). Search terms and strategies were refined according to the requirements of each database, and advanced filters were used. Two independent authors have carried out the search process and the discrepancies have been resolved by discussion.

Eligibility criteria were evaluated using the PICO guidelines. That is population (P): Animal models with ligament or tendon injuries requiring healing interventions. Intervention (I): Hyperbaric oxygen therapy alone or in combination with other treatments (e.g., platelet-rich plasma, rehabilitation exercises, and growth factors), Comparators (C): No HBOT (i.e., no treatment, standard rehabilitation, or alternative therapies without hyperbaric oxygen therapy) and outcome (O): Evaluation of healing outcomes, including biomechanical, histopathological, radiological, biochemical, functional, and subjective measures specific to the injury type.

Inclusion criteria

Studies that involve animal models or humans having ligament or tendon injuries that require healing interventions, including but not limited to anterior cruciate ligament (ACL) injuries, Achilles tendon ruptures, and atrophic tibial nonunion were included. Also, the studies that evaluated the use of HBOT alone or combined with other treatments, such as platelet-rich plasma,

rehabilitation exercises, or growth factors, were included. Studies like randomized controlled trials, prospective cohort studies, non-randomized controlled trials, experimental animal studies and retrospective cohort studies have been included. The studies that had reported the outcome measures that were related to healing outcomes such as biomechanical assessments (e.g., tensile strength, stiffness), histopathological evaluations, radiological imaging (e.g., MRI, X-ray), biochemical analyses (e.g., collagen synthesis), functional assessments (e.g., range of motion, joint stability), and subjective measures (e.g., pain scores, patient-reported outcomes) were also included in this systematic review.

Exclusion criteria

Studies that are not relevant to ligament and tendon injuries were excluded. Also, the studies that evaluate HBOT for indications other than ligament or tendon injuries and insufficient data were excluded. Duplicate studies or overlapping studies were excluded. Studies with a high risk of bias or methodological limitations like inadequate sample size, lack of control group, and studies conducted on healthy subjects without ligament or tendon injuries were removed. Animal studies not relevant to the human condition are also excluded.

Data extraction

Two independent researchers and Excel spreadsheets have carried out the data collection have been used for data extraction. The extraction information includes the name of author, year of study, study design, sample size, study population, control, intervention, and outcomes. Specific variables like Biomechanical outcomes (Maximum force, elastic modulus, etc.), Histological outcomes, Radiological Outcomes, Biochemical Outcomes (Gene expression), Functional Outcomes (functional scores, time to return to play, range of motion, etc.) were also collected for analyzing the efficacy of the HBOT treatment method.

Quality assessment

The quality assessment was carried out by Cochrane’s Risk of Bias in Non-randomized Studies (ROBINS-1) tool [10]. The tool's items have been classified into seven domains, such as random sequence generation, allocation concealment, participants and personnel blinding, outcome

assessment blinding, incomplete outcome data, selective reporting, and other biases. Each domain in the included studies has been assessed as low, moderate, serious, critical, and no information.

RESULTS

Screening literature and results

Figure.1 illustrates PRISMA flowchart for process and results in the screening of literature. The screening process was carried out as per the PICO (Population, Intervention, Comparison, Outcome, and Study Design) principle. Total of 2501 articles were identified from 6 databases. After excluding the duplicates there were 495 articles and irrelevant articles (1105), 901 articles were initially screened. Then, the 496 articles were removed during the screening of the title and abstract because they were not related to the review and were not available in full-text articles. Then a total of 405 relevant articles were screened and 366 articles were excluded after studying full-text articles. In the end, 19 articles were assessed for their eligibility, and 5 articles were removed as they have no expected outcome of interest. Finally 13 studies [8][11][12][13][14][14][15][16][17][18][19][20][21][22] were included as they were eligible and their quality has been assessed. Additionally, the modes of intervention were different among the 14 selected studies, and it may affect the efficacy of HBOT. so, we collected the data on HBOT parameters like study duration, pressure used, and the variables measured during research, which is given in Table 2.

Basic information about the included studies

A total of 693 participants have been used in the 13 studies. Of the 13 studies, 12 studies were experimental [8][12][13][14][15][16][17][18][19][20][21][22], and 1 clinical trial [11] has been used in this systematic review. In 7 studies 2.5 ATA pressure has been used in hyperbaric chamber [12][13][15][18][19][21][22], 1.3 ATA in 1 study [14], 2.4 in 1 study [17], 2.8 ATA in 2 studies [14][20], in one study 4 groups have been analyzed separately in that 2 groups used 2 ATA and one group used 1.5 ATA [16] and in 1 clinical trial study pressure range of 2.0 to 2.5 ATA [11] has been used. In 9 studies HBOT is compared with control, in one study HBOT was used with platelet growth factor-bb [13] to check its efficacy as support treatment, in one study HBOT was treated alongside intermittent oxygen therapy [16], 1 study has HBOT as adjuvant therapy [8], 1 study has combined steroid injection and HBOT treatment [17] and 1 study has used HBOT along

with platelet-rich plasma [22] for analyzing the feasibility of fastest recovery. The efficacy of the HBOT has been measured by different measurements in different studies, as most of the parameters were different in each study. The comprehensive details regarding the measured variables have been given in Table 2 and the outcomes of each study have been given in Table 1.

Quality assessment- Risk of Bias (ROBINS-I)

The risk of bias was analyzed for all fourteen research studies using ROBINS I. ROBINS I focuses on analyzing non-randomized control trials with interventions. Hence, this review utilized ROBINS I to determine the risk of bias. There are a total of seven domains and around 95% have a low risk of bias. This means that the intervention, outcome, and information are clear and present. No confounding was seen in all the papers. However, Enokida et al [11] have a certain serious bias as the research is ongoing and the outcome cannot be determined with relevance to intervention. Two papers [20][22] had the same missing information and were marked seriously. However, overall bias is not changed as the desired outcome is met despite missing information. Overall, the risk of bias determined using ROBINS I has satisfactory results on the utilization of the review for further studies in the future. The risk of bias is given in Figures 2 and 3.

Discussion

The overall study findings in this systematic review are based on the 13 studies that involve 693 participants including animals and humans affected by ligament or tendon injuries. And they were treated with HBOT individually or as a complementary treatment method, for analyzing if HBOT has improved healing the injuries. It can be observed that either as a complementary treatment or individual treatment, HBOT has shown improvement in the healing of injuries compared to control in most of the studies. The pressure used in the hyperbaric chamber varied across studies from 1.3 to 2.8 ATA. A radar chart illustrating the interventions of the first 12 studies and comparative outcomes by the Likert Scale is depicted in Figure 4. The Likert-based qualitative ratings of mechanical and histological outcomes are plotted, whereby a rating of ‘5’ is excellent, ‘3’ is good, and ‘1’ is poor.

This systematic review identified various experimental studies that used HBOT as a treatment method. Almost all studies have analyzed the outcome variables like functional, histological, biomechanical, biochemical, and radiological outcomes. Chan et al [13] have studied the efficacy

of HBOT and platelet growth factor-bb, as a combined method as well as individual treatment options. The result showed that the combined method has given an optimal result. This study suggested that HBOT can be a potential treatment method for MCL healing. In a study by Oyaizu et al., [23] the rate skeletal muscle injury model has been used. It has been reported that HBOT therapy can reduce muscle weight, extracellular space and vascular permeability which results in the reduction of edema. Regarding the HBOT effects on healing of ligament injury, various animal studies have been identified. Horn et al., [14] have used rat models for surgical MCL laceration and treated with HBOT. Here maximum failure load and stiffness in the fourth week have been observed in HBOT groups. This research suggested that the HBOT has accelerated the treatment process and made the ligament return to normal faster. Mashitori et al., [18] also treated the MCL in rat models and applied HBOT for 5 days. The maximum failure load and type I collagen gene expression have been noted. This shows that HBOT has helped accelerate the healing process. The clinical reports regarding the MCL injury treatment using HBOT are very scarce. Soolsma [24] has reported the HBOT effects on functional recovery after the injury with the help of a double-blind controlled study. However, this study was published as a university report and not as the original article. The results by Yagashita et al. [20], a clinical study, examined the grade 2 MCL injury that happened during sports activities. Here the HBOT has been identified to have short-term effects if used for pain reduction in the acute phase. Also, the long-term effect can be the acceleration of recovery in a reduced period for return to play.

After the ACL reconstruction, graft rejection may be possible, and the rates range from 0.7 percent to 24 percent. This graft rejection may occur for various reasons. So the success in the ACL reconstruction is mainly based on the healing of grafts Fu et al., [25]. In the study by Leite et al., [8] it is identified that the use of the HBOT treatment method can help reduce graft rejection and enhance the healing process.

Best et al. [26] conducted a study to analyze any improvement in functional recovery and morphological recovery by using HBOT for muscle stretch in the tibialis anterior muscle. They used a rabbit model, and after 7 days of treatment, they identified that the administration of HBOT increased the recovery pace faster than the control group. In the same way, the study by Chan et al [12] used the rabbit model to treat medial collateral ligament healing using HBOT. They have reported that HBOT has a positive effect on treatment.

Usage of HBOT has been increased especially for the treatment of chronic wounds. The fundamentals of wound healing include the synthesis of oxygen-dependent collage, proliferation of fibroblast, and angiogenesis. In the regeneration of the tendon, the fibroblasts can produce collagen, proteoglycan, and protein mediators Wang et al., [27]. In the study by Hsu et al., [15] the histological results showed an increase in the production of mature fibroblast. This provides a positive effect of HBOT treatment on the tendon healing process. In the study by Ishii et al., [28], the treatment using HBOT has increased the pace of ligament healing at the molecular level with the treatment once daily. At the same time, the results of Ishii et al., [16] have reported that HBOT treatment was effective even for the post-injury recovery process. Also, intermittent HBOT treatment has given a positive result. This can be attributed to the fact that HBOT promotes angiogenesis and muscle regeneration by increasing levels of nitric oxide (NO), vascular endothelial growth factor, and basic fibroblast growth factor [29].

HBOT can play a role in the postoperative management of ligament injuries, such as in preventing graft failure and re-injury in patients with an ACL reconstruction. Although maintaining a robust rehabilitation schedule is critical to promote graft ligamentization, rates of graft failure and re-injury are still substantial. It is estimated that at least 10 percent of ACL grafts get ruptured, resulting in a significant financial and emotional burden for patient and patient care team [30]. In recent years, there are various experimental therapies being explored to improve the graft healing, such as delivering photopolymerized hydrogels or biologics like stem cells at the graft site [31]. However, the complexity, cost, and variability in their outcomes have limited their application in the current environment. The systematic review reveals how HBOT provides a generalized enhancement of the healing environment, which could potentially lead to a more consistent and widespread benefit in ligamentization.

In the context of complete tendon ruptures and degenerative tendon disease, HBOT can also serve as an adjunct rehabilitative modality. Tendon repair at the molecular level can be understood in three phases: the inflammatory phase, proliferative phase, and consequently, the remodeling phase [32]. The inflammatory phase is triggered by the insult and is orchestrated by pro-inflammatory cytokines such as IL-6. The proliferative phase involves the activation of tenocytes to allow for the synthesis of collagen and other components of the extracellular matrix, and the final remodeling phase is completed by the maturation and alignment of collagen fibers. The enhanced

oxygen supply offered by HBOT can help drive the inflammatory and proliferative phases, and its ability to modulate inflammation can simultaneously prevent poor healing outcomes.

This systematic review mainly highlights the benefits of HBOT for ligament and tendon injuries but also has some limitations. This review includes both animal and human studies. The animal studies provide valuable information regarding HBOT in ligament and tendon injury, but it is difficult to generalize them to human treatments. Each study has used different HBOT parameters that include pressure, duration of treatment, and frequency. This inconsistency makes it difficult to compare their effectiveness in HBOT and determine the optimized protocol.

This review has identified that high-quality clinical trials are scarce in the HBOT for ligament injuries like MCL tears. Among existing studies, a pilot study [33] investigated the short-term effects of HBOT on athletes with acute ankle sprains. Although there was no control group, the study revealed that HBOT significantly reduced foot and ankle volumes during activity and rest and provided substantial pain relief within 3-4 sessions. There is a need for more clinical trials to confirm the animal studies' findings and assessment of long-term effects on humans.

The limitations analyzed in this systematic review suggest the need for future research to determine the HBOT's role in treating ligament and tendon injuries. There is a need to address the need for a standardized protocol for the HBOT, more high-quality clinical trials with a larger population, and research on the long-term effects of HBOT on the healing of ligament and tendon injuries that includes the functional outcomes and potential side effects.

CONCLUSION

This systematic review mainly examines the efficacy and safety of hyperbaric oxygen therapy for ligament and tendon injuries. The findings of this study have identified the need for more clinical trials and human studies in HBOT. Also, it can be seen that HBOT has no critical side effects that are life-threatening and are safe to use. The efficiency of the HBOT method has been observed to accelerate the leaving process compared to control. It can be identified that HBOT can be used as a standalone treatment method or as an adjunctive treatment method. The results from all the studies in this review have shown that HBOT can be used for the treatment of ligament and tendon injuries, and they are safe and help in quick recovery.

Reference

1. Musculoskeletal health. Available from: https://www.who.int/news-room/fact-sheets/detail/musculoskeletal-conditions

2. Wu F, Nerlich M, Docheva D. Tendon injuries: Basic science and new repair proposals. EFORT Open Reviews. 2017 Jul ;2(7):332–42. Available from: https://eor.bioscientifica.com/view/journals/eor/2/7/2058-5241.2.160075.xml

3. BMUS: The Burden of Musculoskeletal Diseases in the United States. Bmus: the burden of musculoskeletal diseases in the United states. Available from: https://www.boneandjointburden.org/

4. Leong NL, Kator JL, Clemens TL, James A, Enamoto-Iwamoto M, Jiang J. Tendon and ligament healing and current approaches to tendon and ligament regeneration. Journal Orthopaedic Research. 2020 Jan;38(1):7–12. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jor.24475

5. Aicale R, Maffulli N, Oliva F. Regeneration and repair of ligaments and tendons. In: Human Orthopaedic Biomechanics. Elsevier; 2022 p. 485–99. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128244814000305

6. Su CA, Jildeh TR, Vopat ML, Waltz RA, Millett PJ, Provencher MT. Current state of platelet-rich plasma and cell-based therapies for the treatment of osteoarthritis and tendon and ligament injuries. Journal of Bone and Joint Surgery. 2022 Aug 3;104(15):1406–14. Available from: https://journals.lww.com/10.2106/JBJS.21.01112

7. Hu S li, Feng H, Xi G hua. Hyperbaric oxygen therapy and preconditioning for ischemic and hemorrhagic stroke. Med Gas Res. 2016;6(4):232. Available from: https://journals.lww.com/10.4103/2045-9912.196907

8. Leite CBG, Leite MS, Varone BB, Santos GBD, Silva MDS, Pereira CAM, et al. Hyperbaric oxygen therapy enhances graft healing and mechanical properties after anterior cruciate ligament reconstruction: An experimental study in rabbits. Journal Orthopaedic

Research. 2024 Jun;42(6):1210–22. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jor.25787

9. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021 Mar 29;n71. Available from: https://www.bmj.com/lookup/doi/10.1136/bmj.n71

10. Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ. 2016 Oct 12;i4919. Available from: https://www.bmj.com/lookup/doi/10.1136/bmj.i4919

11. Enokida M. Effect of combined hyperbaric oxygen therapy on anterior cruciate ligament reconstruction. NIPH Clinical Trials Search. Published online March 31, 2022. JRCT ID: jRCT1062210054. Available from: https://rctportal.niph.go.jp/en/detail?trial_id=jRCT1062210054

12. Chan. YS, Lin. SS, Lee. MSS, Chang. CH, Chen. ACY, Ueng. SWN. Effects of Hyperbaric Oxygen and Platelet Growth Factor-BB on Ligament Healing". Poster 2004 Orthopaedic Research Society, San Francisco USA.

13. Chan. YS, Lin. SS, Lee. MSS, Chang. CH, Chen. ACY, Ueng. SWN. Effect of hyperbaric oxygen therapy on medial collateral ligament healing in a rabbit model. Poster 2007 Orthopaedic Research Society, San Francisco USA.

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: Clinical Orthopaedics and Related Research. 1999 Mar;360:238–42. Available from: http://journals.lww.com/00003086-199903000-00028

15. Hsu RWW, Hsu WH, Tai CL, Lee KF. Effect of hyperbaric oxygen therapy on patellar tendinopathy in a rabbit model: The Journal of Trauma: Injury, Infection, and Critical Care. 2004 Nov;57(5):1060–4. Available from: http://journals.lww.com/00005373-200411000-00023

16. Ishii Y, Ushida T, Tateishi T, Shimojo H, Miyanaga Y. Effects of different exposures of hyperbaric oxygen on ligament healing in rats. Journal Orthopaedic Research. 2002 Mar;20(2):353–6. Available from: https://onlinelibrary.wiley.com/doi/10.1016/S0736-0266%2801%2900094-8

17. Kuran FD. Effect of hyperbaric oxygen treatment on tendon healing after Achilles tendon repair: an experimental study on rats. Acta Orthop Traumatol Turc. 2012;46(4):293–300. Available from: https://www.aott.org.tr/en/effect-of-hyperbaric-oxygen-treatment-on-tendon-healing-after-achilles-tendon-repair-an-experimental-study-on-rats-134206

18. Mashitori H, Sakai H, Koibuchi N, Ohtake H, Tashiro T, Tamai K, et al. Effect of hyperbaric oxygen on the ligament healing process in rats. Clinical Orthopaedics & Related Research. 2004 Jun;423:268–74. Available from: https://journals.lww.com/00003086-200406000-00044

19. Takeyama N, Sakai H, Ohtake H, Mashitori H, Tamai K, Saotome K. Effects of hyperbaric oxygen on gene expressions of procollagen, matrix metalloproteinase and tissue inhibitor of metalloproteinase in injured medial collateral ligament and anterior cruciate ligament. Knee Surg Sports Traumatol Arthrosc. 2007 Apr 2;15(4):443–52. Available from: http://link.springer.com/10.1007/s00167-006-0241-4

20. Yagishita K, Enomoto M, Takazawa Y, Fukuda J, Koga H. Effects of hyperbaric oxygen therapy on recovery acceleration in Japanese professional or semi-professional rugby players with grade 2 medial collateral ligament injury of the knee: A comparative non-randomized study. Undersea Hyperb Med. 2019 Dec;46(5):647–54.

21. Yeh W, Lin S, Yuan L, Lee K, Lee MY, Ueng SWN. Effects of hyperbaric oxygen treatment on tendon graft and tendon-bone integration in bone tunnel: Biochemical and histological analysis in rabbits. Journal Orthopaedic Research. 2007 May;25(5):636–45. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jor.20360

22. .Zang Y, Zhao X jiang, Wang L, Zhu W fang. Clinical effect of hyperbaric oxygen combined with platelet-rich plasma on anterior cruciate ligament injury reconstruction. Published online 2020. Accessed February 6, 2024.

https://www.semanticscholar.org/paper/Clinical-effect-of-hyperbaric-oxygen-combined-with-Zang-Zhao/1542dfb4c413339203535691ed00bc931ccb1d0a

23. Oyaizu T, Enomoto M, Yamamoto N, Tsuji K, Horie M, Muneta T, et al. Hyperbaric oxygen reduces inflammation, oxygenates injured muscle, and regenerates skeletal muscle via macrophage and satellite cell activation. Sci Rep. 2018 Jan 22;8(1):1288. Available from: https://www.nature.com/articles/s41598-018-19670-x

24. Soolsma SJ. The effect of intermittent hyperbaric oxygen on short term recovery from grade II medial collateral ligament injuries. University of British Columbia; 1996 Available from: https://open.library.ubc.ca/soa/cIRcle/collections/ubctheses/831/items/1.0077081

25. Fu FH, Shen W, Starman JS, Okeke N, Irrgang JJ. Primary anatomic double-bundle anterior cruciate ligament reconstruction: a preliminary 2-year prospective study. Am J Sports Med. 2008 Jul;36(7):1263–74. Available from: http://journals.sagepub.com/doi/10.1177/0363546508314428

26. Best TM, Loitz-Ramage B, Corr DT, Vanderby R. Hyperbaric oxygen in the treatment of acute muscle stretch injuries. Am J Sports Med. 1998 May;26(3):367–72. Available from: http://journals.sagepub.com/doi/10.1177/03635465980260030401

27. Wang MX, Wei A, Yuan J, Clippe A, Bernard A, Knoops B, et al. Antioxidant enzyme peroxiredoxin 5 is upregulated in degenerative human tendon. Biochemical and Biophysical Research Communications. 2001 Jun;284(3):667–73. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0006291X01949915

28. Ishii Y, Miyanaga Y, Shimojo H, Ushida T, Tateishi T. Effects of hyperbaric oxygen on procollagen messenger rna levels and collagen synthesis in the healing of rat tendon laceration. Tissue Engineering. 1999 Jun;5(3):279–86. Available from: https://www.liebertpub.com/doi/10.1089/ten.1999.5.279

29. Yagishita K, Oyaizu T, Aizawa J, Enomoto M. The effects of hyperbaric oxygen therapy on reduction of edema and pain in athletes with ankle sprain in the acute phase: A pilot study. Sport Exerc Med Open J. 2017; 3(1): 10-16. doi: 10.17140/SEMOJ-3-141

30. Rousseau R, Labruyere C, Kajetanek C, Deschamps O, Makridis KG, Djian P. Complications After Anterior Cruciate Ligament Reconstruction and Their Relation to the Type of Graft: A Prospective Study of 958 Cases. Am J Sports Med. 2019;47(11):2543-2549. doi:10.1177/0363546519867913

31. Chen CH. Graft healing in anterior cruciate ligament reconstruction. BMC Sports Science, Medicine and Rehabilitation. 2009;1(1):21. doi:10.1186/1758-2555-1-21

32. Dyment NA, Galloway JL. Regenerative Biology of Tendon: Mechanisms for Renewal and Repair. Curr Mol Bio Rep. 2015;1(3):124-131. doi:10.1007/s40610-015-0021-3

33. Yamamoto, N., Oyaizu, T., Enomoto, M. et al. VEGF and bFGF induction by nitric oxide is associated with hyperbaric oxygen-induced angiogenesis and muscle regeneration. Sci Rep 10, 2744 (2020). https://doi.org/10.1038/s41598-020-59615-x

Figure Legends

Table 1: Study characteristics of included articles in systematic review

Table.2 The modes of HBOT intervention and variable measured (outcomes) in 14 articles

Figure 1: PRISMA flow diagram

Figure 2 : Traffic Light Signal on individual studies included in this review

Figure 3: Overall Summary on risk of bias of HBOT

Figure 4: Likert Scale Radar Chart Across Studies.

Table 1. Study characteristics of included articles in systematic review

Author	Year	Study design	Human/ Animal?	Sample Size	Study population	Control	Intervention	Outcomes
Enokida et al [11]	2022	Clinical Trials (ongoing)	Human	80	Men and Women aged 14 to 45.	Does not undergo HBOT	Hyperbaric oxygen (HBOT) therapy. (2.0-2.5 ATA for 60-120 minutes)	Maturation of anterior cruciate ligament evaluated by MRI. quantitative evaluation, physical findings, knee function and safety will be evaluated.
Chan et al [12]	2004	Experimental research using a rabbit model.	Animal	64 (control:32 and experimental: 32)	Rabbits (specific age and weight were not mentioned). Gender: Male.	Does not undergo HBOT	HBOT at 2.5 atmosphere for 2 hours everyday	Displacement-time curve: increases at the rate of 7.2 mm/min constantly. Control : Intact left medial collateral ligament, the mean percentage of failure load in HBOT group at 2 weeks: 20.6%, 4: 49.1%, 8: 63.9%, and 12 weeks:76.3%. For non-HBOT groups, 2 weeks:13.5%, 4 weeks:25.3%, 8 weeks: 36.5%, and 12 weeks:57.3%. Histology analysis: HBOT group had collagen fibres at higher density and better alignment than non-HBOT group.
Chan et al [13]	2007	Experimental study on platelet growth factor and hyperbaric oxygen for the treatment of BB healing.	Animal	MCL fibroblasts	New Zealand white rabbits (specific ages and genders not mentioned).	Cells maintained in 5% CO2 / 95% air.	Hyperbaric oxygen (HBOT) therapy	HBOT when treated increased the MCL cell count than controlled cells. PDGF-bb also increased the cell count of MCL when treated dose dependently compared to controlled cells. Type one PDGF-bb combined with HBOT treatment, H/C ratio was 101.6% ± 4.1%, P/C ratio was 99.7% ± 4.7%, HP/C ratio was 103.8% ± 3.2%. Type three collagen after HBOT or HBOT and PDGF-bb decreased the collagen by H/C

								ratio was 90.6% ± 2.1%, P/C ratio was 100.7% ± 5.7%, HP/C ratio was 92.3% ± 2.2%.
Horn et al [14]	1999	Experimental study on understanding hyperbaric oxygen effect on healing of rat model's ligament.	Animal	48 Sprague-Dawley rats.	Sprague-Dawley rats, No gender mentioned	Controls: 24 rats recovered without intervention.	Hyperbaric oxygen (HBOT) therapy	At 4 weeks, ligaments exposed to HBOT needed a higher statistical force to cause failure compared to those not exposed to HBO, indicating enhanced healing. At the sixth week there were no additional statistics in which the increase of force or stiffness was observed. This suggests that four weeks are necessary for ligament healing.
Hsu et al [15]	2004	Experimental research on use of hyperbaric oxygen therapy on patellar tendinopathy in rabbit models.	Animal	13 New Zealand male rabbits.	The study population contains a four month old male rabbit with three kg.	The left knee of each rabbit served as a control, remaining intact.	Hyperbaric oxygen (HBOT) therapy	HBOT effectively enhances the healing of tendinopathy by increasing cross-linking and synthesizing collagen. The collagen tensile load had HBOT was 34.8% higher than the tendon control at 10 weeks.. About 82.2% of hydroxyproline concentrations increase the hyperbaric oxygen therapy. Hyperbaric oxygen therapy indeed provides validated results in tendinopathy treatment.
Ishii et al [16]	2002	Experimental study on utilizing HBOT for healing of ligament	Animal	44	44 male Wistar rats that are eight weeks old and weigh between 250-270g are considered.	Control Group (Group A): Normobaric room air at one atmosphere absolute for one hour.	Hyperbaric oxygen therapy	The outcomes prove that HBOT can effectively heal tendon repairs in comparison with control groups and ATA for sixty minutes have enhanced the deposits of extracellular matrix and increased collagen synthesis.
Kuran et al [17]	2012	Experimental study in determining the healing of	Animal	Fifty-six male Wistar albino rats	The study population contains twenty four rats from each	1.Group 1: Tendon repair without any treatment 2.Group 2: HBOT	Hyperbaric oxygen (HBOT) therapy	Hyperbaric oxygen therapy has increased the fibrosis volume and neovascularization shows the histopathology study. Biochemical differences were seen in all the groups. Additionally, variation was

		achilles tendon repair using hyperbaric oxygen treatment			population. Within age of five to seven months, male, and weighing between 200-260 grams	therapy after tendon repair 3. Group 3: Repair of tendon after injection with steroids. 4. Group 4: Repair of tendon and treatment of HBOT HBOT after injection of steroid.		observed in higher levels considering inflammation and vascularization after steroid administration. HBOT was able to heal Achilles tendon repair.
Leite et al [8]	2024	ACL reconstruction rabbit model	Animal	Twelve New Zealand rabbits, male and matured skeletally.	Rabbits that undergo reconstruction of ACL with or absence of adjuvant HBOT weighing around 2.8 kg.	Ambient air group (n = 6), and placed in normal air till the entire time.	HBOT group (n = 6), exposed to one hundred percent oxygen at 2.5 ATA, for five days with two hour daily treatment beginning after the first day of surgery.	HBOT improved the Anterior cruciate ligament graft mutation and integration. The HBOT has also enhanced biochemical properties, widened, and reduced the tunnel of the graft. This paves way for the research in future.
Mashitori et al [18]	2004	Experimental study on rats	Animal	Male sex rats of spargue-Dawley with a count of seventy six.	Sprague-Dawley rats (eleven weeks old rat weighing between 369 to 9 g)	Room air group (Group C): 38 rats exposed to room air	HBOT group (Group H): 38 rats, HBO:t 2.5 ATA, 2 hours, 5 days in a week.	Weight gain in rats exposed to hyperbaric oxygen (Group H) was lesser compared to the control group. (Group C) at 3, 7, 14, and 28 days postoperatively, with significant differences observed at 7, 14, and 28 days . Macroscopic inspection showed that compared to Group C, Gropp H has a higher scar tissue rate, with the most significant difference observed at 7 days postoperatively. Histologic findings showed that cells of inflammation are dominant in scar tissue three days postoperatively, transitioning to active fibroblasts dominating the tissue by 2 weeks. Compared to Group C at 7 and 14 days postoperative the Type I procollagen are

								expressed maximum in Group H , indicating a positive hyperbaric oxygen therapy effect on expression of genes. At Group H the healing and stiffness of tendons are higher compared to Group C in fourteen days.
Takeyama et al [19]	2007	Experimental study	Animal	128 male Sprague-Dawley rats	Sprague-Dawley rats , 11 weeks old, weight 356 ± 14 grams	Normal atmospheric pressure groups for MCL and ACL injuries (Groups MC and AC, respectively)	HBOT groups for MCL and ACL injuries (Groups MH and AH, respectively), 100% oxygen 2.5 ATA for 2 hours for 5 days in a week	HBOT administration increased type I procollagen gene expression in MCL and ACL injury. Tissue inhibitors of metalloproteinases (TIMPs) gene expression, increased after operation in 2 groups. MMP gene expressions were not affected by HBO. Injured MCL, TIMP gene expressions were not affected, and in injured ACL, increase in TIMP gene expressions. Type I procollagen expression in injured MCL increased with HBOT on the 7th day. Injured ACL has increased expression type I procollagen than normal ACL, with HBOT increasing gene expression at 28 days after injury.
Yagashita et al [20]	2019	Experimental study	Human	32 professional or semi-professional rugby players, with grade 2 MCL knee injury HBOT group =16, non-		HBOT group (n=16), non-HBOT group (n=16)	2.ATA (283.6 kPa) for 60 minutes, Each patient received five HBOT treatments in 10 days after injury.	VAS scores at pain at rest immediately before: 18.8 ± 17.7 and after: 17.3 ± 16.4, HBOT therapy on the same day (p=0.11). pain while walking, before: 37.4 ± 20.1 and after: 32.4 ± 21.8, HBOT therapy (p<0.001). pain while jogging, before: 50.7 ± 25.6 and after: 43.9 ± 25.0, HBOT therapy. Time to return to play: 31.4 ± 12.2, 10-58 days in HBOT group and 42.1 ± 15.8, 18-71 days in non-HBOT group.

				HBOT group=16.				Early or late HBOT application, time to return to play in early HBOT group was 27.9 ± 9.3, 10-41 days within two days after injury (n=11) and in the delayed HBOT group, 39.0 ± 13.1 23-58 days three to five days after injury (n=5). No significant differences.
Yeh et al [21]	2007	Experimental study on HBOT on tendon integration and grat in bone tunnel	Animal	Forty rabbits	40 New Zealand rabbits that weighs 3.2 to 1.6 kg were utilised	The control group *n=20* is caged and exposed to normal air	Hyperbaric Oxygen Treatment	The electronic microscope showed that regeneration of collagen fibres have increased after HBOT treatment. Hence, after ACL surgery HBOT can potentially improve the results.
Zang et al [22]	2020	Experimental study	Human	100 human patients with ACL (control:50 and experimental: 50)		Control group only treated with PRP	Treated with hyperbaric oxygen combined with PRP	Total effective rate: 90.0%, was higher than the control group (68.0%) ($P<0.05$). Scores of Lysholm, Tegner, and IDKC were higher compared to the control group ($P<0.05$). The contents of IGF-1, BGP, and MMP-1 were less than before treatment, and lower than the control group ($P<0.05$). HBOT combined with platelet-rich plasma can improve the therapeutic effect of ACL reconstruction, improving the serum contents of IGF-1, BGP, and MMP-1.

Table.2 The modes of HBOT intervention and variable measured (outcomes) in 14 articles

Author	Treatment	Pressure	Treatment time	Variables measured	Details of measured variables
Enokida et al [11]	HBOT	2.0-2.5 ATA	60-120 minutes	Functional Outcomes	Knee joint swelling, muscle atrophy, range of motion, anterior movement amount, half-month movement amount over time
Chan et al [12]	HBOT	2.5	2 hours, daily	Histologic analysis	Density and alignment of collagen fibers in healing
				Biomechanical outcomes	Force and displacement at tensile tests
Chan et al [13]	HBOT and Platelet growth factor-bb	2.5 ATA	120 minutes per 48 hours	Biochemical analyses	Type I collagen and Type III Collagen synthesis
Horn et al [14]	HBOT	2.8 ATA	1.5 Hours a day for 5 days	Biomechanical outcomes	Force And displacement
Hsu et al [15]	HBOT	2.5 ATA	120 minutes for 10 Weeks	Mechanical analysis	Tensile load
				Biochemical analysis	Hydroxyproline, pyridinoline,
				Histological analysis	Vascularity
Ishii et al [16]	HBOT and intermittent oxygen	Group B:1.5 Group C:2 Group D:2	Group B:30 min daily. Group C:30 min daily. Group D:60 min daily.	Histological analysis	Ligament healing
				Biochemical analysis	Pro-a1 (I) mrna expression
Kuran et al [17]	Steroid injection and HBOT therapy	2.4 ATA	70 minutes per day for 7 days	Histologic analysis	Inflammatory Cells, veins and fusiform fibroblast cells
				Biomechanical outcomes	Tensile behaviors Like rigidity, elastic modulus and energy absorption density, Rupture loads, maximum loads)
Leite et al [8]	HBOT (Adjuvant)	2.5 ATA	2 h daily for 5 days	Radiological imaging	Magnetic resonance imaging (MRI), or high-resolution peripheral Quantitative computed tomography (HR-pqct) scan

				Biomechanical analysis	Tension force (force, stiffness, and elongation of ligament rupture)
Mashitori et al [18]	HBOT	2.5 ATA	2 h for 5 days	Biomechanical analysis	Tensile failure (ultimate load and Stiffness)
				Biochemical analysis	Type I procollagen gene expression
Takeyama et al [19]	HBOT	2.5 ATA	2 h for 5 days	Biochemical analysis	Gene expressions of procollagens, matrix metalloproteinases (mmps) and mmps tissue inhibitors
Yagashita et al [20]	HBOT	2.8 ATA	60 minutes, 5 treatments in 10 days	Functional Outcomes	VAS scores, Time to return to play, pain reduction
Yeh et al [21]	HBOT	2.5 ATA	2 h for 5 days	Histological analysis	Tendon-bone interface
				Biomechanical analysis	Tension strength
Zang et al [22]	HBOT and platelet-rich plasma	-	-	Biochemical analysis and Functional Outcomes	The clinical efficacy, knee joint, Lysholm, Tegner, IDKC scores, serum insulin-like growth factor-1 (IGF-1),osteocalcin (BGP) and matrix metalloproteinase-1

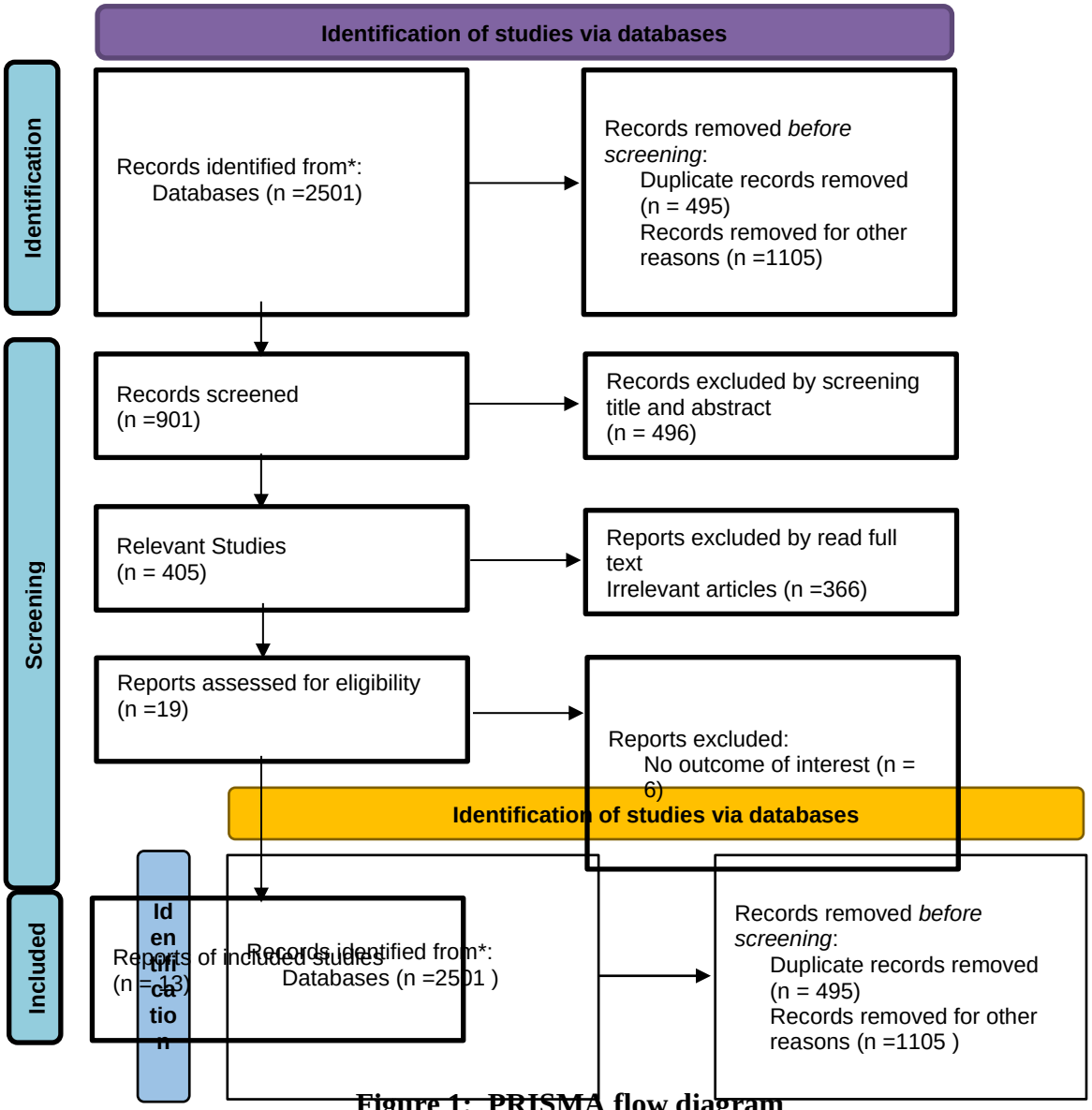

Figure 1: PRISMA flow diagram

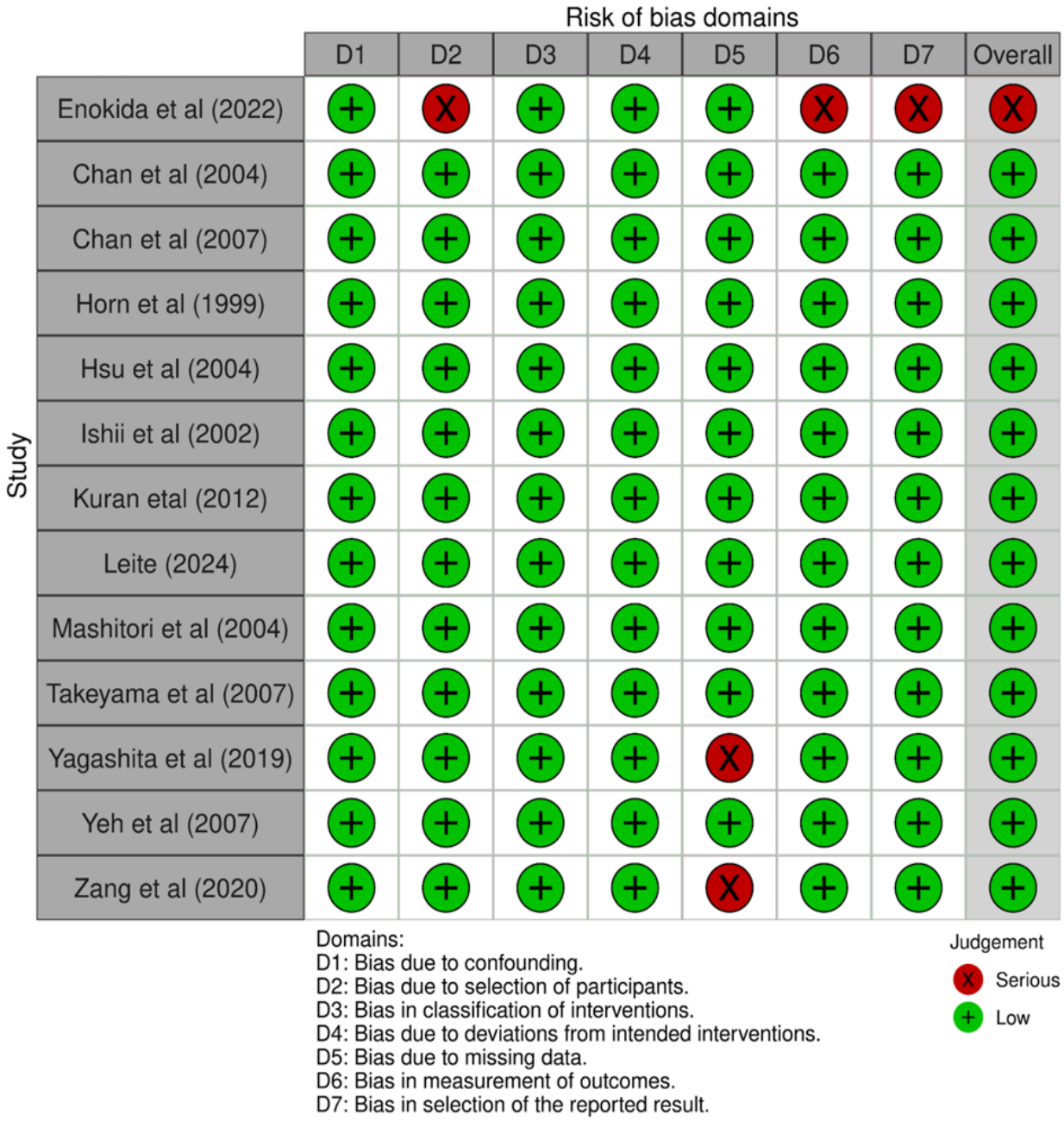

Figure 2. Traffic Light Signal on individual studies included in this review

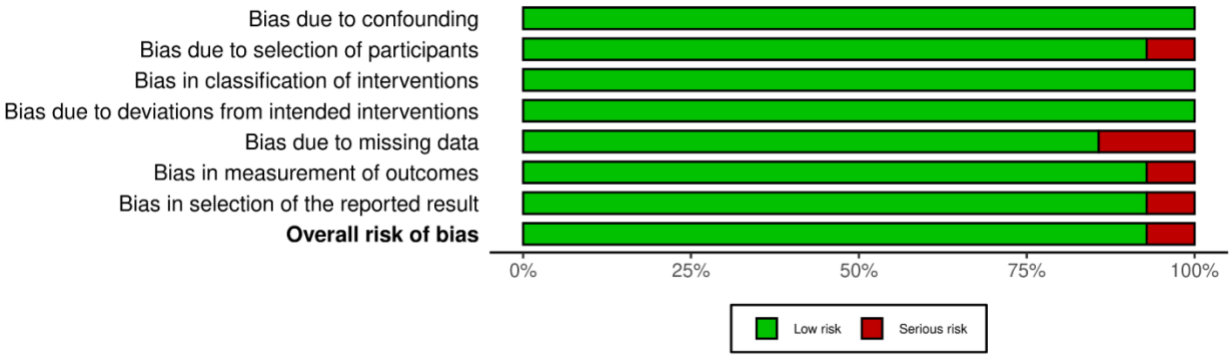

Figure 3. Overall Summary on risk of bias of HBOT

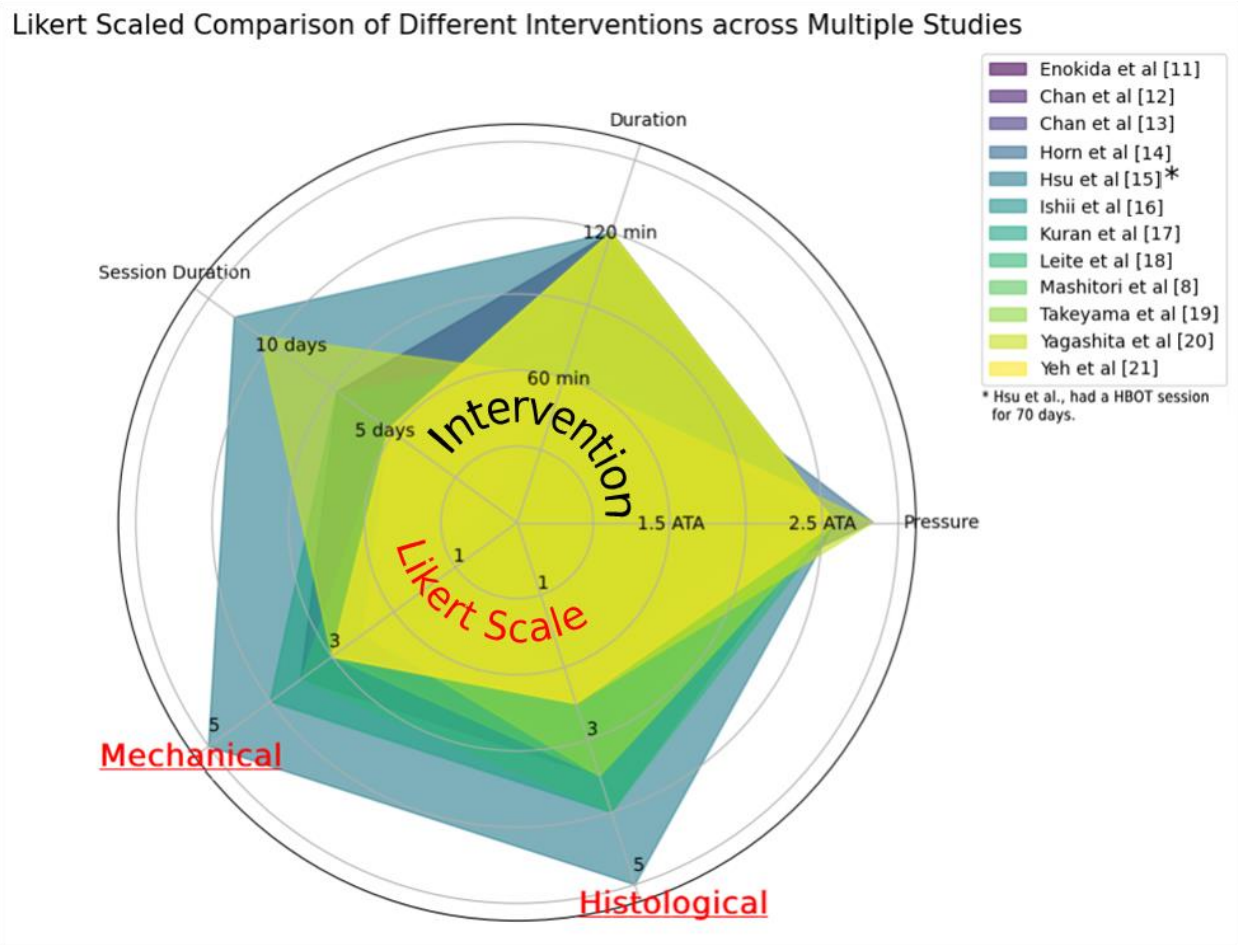

Figure 4. Likert Scale Radar Chart Across Studies. The details of the study interventions are plotted (e.g., HBOT session duration, duration, and pressure) and the Likert-based qualitative ratings of mechanical and histological outcomes are plotted. A rating of '5' is excellent, '3' is good, and '1' is poor.