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Medical Gas Research

journal homepage: www.medgasres.com

A scoping review and evaluation of hyperbaric oxygen therapy for skeletal muscle injury in preclinical models

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https://doi.org/10.4103/mgr.MEDGASRES-D-24-00163

Date of submission: December 31, 2024

Date of decision: February 18, 2025

Date of acceptance: April 18, 2025

Date of web publication: August 18, 2025

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Abstract

Although hyperbaric oxygen therapy (HBOT) is promising for the alleviation of limb trauma or crush muscle injuries, critical examination of the state of related science is lacking. We conducted a scoping review and evaluation of HBOT on muscle injury in preclinical models. A search of PubMed and Web of Science databases yielded 157 reports published from the start of the databases until November 7, 2024, which narrowed to 19 after removing duplicates, non-muscle studies, and dissertations/reviews. The studies involved mice or rats treated with tourniquets or exposed to a myotoxic agent (bupivacaine and cardiotoxin) or crush to induce muscle injury. HBOT counteracted metabolic effects and had differential effects on oxidative stress in the tourniquet model. Overall, HBOT promoted or quickened muscle regeneration initiated by myotoxic agents and crush. These findings also indicate that HBOT benefits may persist, and early initiation of HBOT is important. However, more sessions do not always yield better outcomes. The evaluation of the state of the science revealed that the inclusion of females in these studies is limited, and milder pressure levels have been undertested, which may be important for fewer adverse effects and access. Future research in these and other areas may lead to increased use and acceptability of HBOT for the treatment of limb trauma or crush muscle injuries.

Key Words: bupivacaine; cardiotoxin; contusion; crush; muscle regeneration; pressure levels; rodents; sessions; sex differences; tourniquet

Introduction

Limb trauma is a common clinical condition involving soft tissue damage, fractures, or both. A crush injury represents one cause of limb trauma. The underlying pathology of crush injury—such as internal bleeding, blood vessel disruption, and temporary or prolonged ischemia-reperfusion—and the consequence of compartment syndrome depend on the properties of the initial insult as well as other factors. In addition, these consequences result in compromised tissue oxygenation.[1] With less oxygen delivered to the tissue, the risk of cell dysfunction or death is likely. One strategy to minimize this risk is to increase the capillary-to-tissue gradient of the partial pressure of oxygen. Delivering pure oxygen under greater atmospheric pressure, as with hyperbaric oxygen therapy (HBOT), can increase the partial pressure of oxygen capillary-to-tissue gradient from 60 mmHg to more than 1900 mmHg.[2] This larger gradient increases the perfusion distance into the tissues, which helps maintain cell viability. For this reason, treating patients who have sustained an extremity crush injury with hyperbaric oxygen seems the best course of action to promote recovery.[1]

Despite its benefits, HBOT is infrequently used for limb trauma/crush injury.[1] One reason is the absence of equipment at major trauma centers. HBOT administration, especially at pressures > 1.4 atmospheres absolute (ATA; 1.0 ATA = 101.325 kPa), requires a large chamber operated by highly trained staff to treat multiple patients simultaneously.[2,3] Other reasons for HBOT's infrequent use include difficulty with third-party reimbursement,[1] clinicians' lack of knowledge about this therapy,[1] and potentially a limited understanding of how HBOT acts at the cellular level in response to crush muscle injury as these data in the biomedical literature are sparse.

In the last 19 years, two systematic reviews involving HBOT and limb injuries have been published.[4,5] Although positive outcomes of HBOT are described for these conditions, including improved limb integrity or intactness, enhanced wound healing, less pathophysiology, and fewer surgical procedures, the results of these clinical studies or cases cannot elucidate HBOT's molecular and cellular activities within the muscle. Preclinical findings are the best source for understanding muscle's molecular and cellular responses to HBOT.

To our knowledge, a critical examination of the state of the science related to preclinical muscle injury and HBOT has not been conducted. Therefore, this article describes the results of a scoping review on this topic. The following research question guided the review: What is known from the literature about HBOT effects on muscle injury and recovery in rat and mouse studies? Also, we evaluate these results and offer recommendations to strengthen the clinical translation of HBOT preclinical muscle injury research to promote the use of HBOT for the treatment of limb trauma or crush muscle injuries.

Methods

Reports that focused on tourniquet-related injury were included in this review because this type of injury could represent the effects of prolonged ischemia related to a crush injury. Studies in which an acute muscle injury was induced by bupivacaine and cardiotoxin were included because these myotoxic agents activate muscle regeneration, which is the vital host response to an acute muscle injury. Other inclusion criteria consisted of English language research reports involving (a) the administration of one or more hyperbaric oxygen treatments to rats or mice and (b) skeletal muscle as an injury target. We searched PubMed and Web of Science databases from the start date of each database. The last search date was conducted on November 7, 2024. The 10 search strategies were as follows: "crush injury" HBO animal; "crush injury" "hyperbaric oxygen" animal; contusion HBO animal; contusion "hyperbaric oxygen" animal; HBO tourniquet animal; "hyperbaric oxygen" tourniquet animal; HBO bupivacaine animal; "hyperbaric oxygen" bupivacaine animal; HBO cardiotoxin animal; and "hyperbaric oxygen" cardiotoxin animal. A total of three reviewers independently conducted the searches, and the three reviewers agreed to the final list of included studies. One reviewer created a spreadsheet in which columns were labeled with key variables for each muscle injury type. A second reviewer verified the data in these spreadsheets.

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How to cite this article: *Schneider BSP, Zhang L, Dombrowsky T, Wilson JR. A scoping review and evaluation of hyperbaric oxygen therapy for skeletal muscle injury in preclinical models. Med Gas Res. 2026;16(2):125-132.*

In addition, one reviewer created tables based on these spreadsheets, and two other reviewers verified these data. If discrepancies were identified, the reviewers discussed and resolved the discrepancies. From each study, data related to the animals (e.g., biological sex and body mass), HBOT (e.g., pressure levels, duration, sessions per day), and major muscle-related outcomes (e.g., oxidative stress/antioxidants, contractile properties, and regeneration) were collected. The spreadsheets and tables directed our synthesis of the results, and these tables are included (**Tables 1–3**).

Results

Figure 1 shows the flow diagram[6] for data selection. A total of 157 articles were identified. After removing duplicates and reports that did not meet the inclusion criteria, we analyzed 9 tourniquet, 4 bupivacaine/cardiotoxin, and 6 crush research reports, totaling 19.

Hyperbaric oxygen therapy on tourniquet-induced skeletal muscle injury

All investigations

In these nine investigations, an elastic or rubber band was applied around a selected thigh to induce ischemia for 1.5–6 hours and then removed to promote reperfusion for up to 48 hours. Most of the studies used male rats (**Table 1**). The use of preemptive or postprocedure opioid analgesia was not mentioned in any of the reports.

The hyperbaric oxygen regimen varied among the nine studies (**Table 1**). In six investigations, HBOT was started after ischemia, which corresponded to the phase of reperfusion.[7-12] In other experiments, researchers started HBOT during the last hour of ischemia[12,13] or before ischemia[14,15]; hyperbaria ranged from 2.0 to 3.0 ATA. Other similarities among most of the studies were oxygen concentration (100%), duration of a single treatment (45 or 60 minutes), and total number of doses or sessions (i.e., one HBOT session). Comparison groups that did not receive HBOT included the following: (a) received ischemia only, (b) received anesthesia only, or (c) sham-operated only. No groups were controlled for pressure (using air only) or normobaric hyperoxia (using only 100% oxygen). Furthermore, no adverse effects associated with HBOT were described in any of the reports.

HBOT effects were measured within an early post-injury period. The shortest endpoint for a tourniquet + HBOT group was 0 minutes postischemia[8]; the longest endpoint for this group was 48 hours postischemia.[11,15] The muscles assayed were located in the hindlimb, including the biceps femoris,[13] gastrocnemius,[15] soleus,[12,14] and tibialis anterior.[7-11] The most commonly observed HBOT effects were metabolic (e.g., adenosine triphosphate, phosphocreatine, and/or lactate levels) or related to oxidative stress/antioxidants (**Table 1**). Overall, HBOT counteracted ischemia-reperfusion's metabolic effects and exhibited differential effects on oxidative stress/antioxidants (**Table 1**). In addition, blood flow improved, and edema, necrosis, and neutrophil presence decreased in response to HBOT (**Table 1**).

Varying initial introduction

Like all treatments, HBOT can vary in multiple parameters, including pressure, initial timing, frequency (number of treatments per day, total sessions, or total days), and duration. Determining the optimal HBOT regimen can involve comparing variations in one or more of these parameters. Among these tourniquet studies, only one group of investigators performed one of these comparisons (i.e., initial timing) and reported that introducing HBOT during both ischemia and reperfusion yielded more positive results than during reperfusion only and ischemia only.[12]

Animals without ischemia

In two experiments, HBOT was administered to animals that did not undergo ischemia.[10,13] No difference in muscle metabolic or oxidative stress/antioxidant parameters was detected in healthy rats with or without HBOT.[11] In another study, oxidative stress/antioxidant parameters were similar between sham-operated (no ischemia) rats that received HBOT and anesthetized-only rats that did not receive HBOT or ischemia.[13] These data suggest that HBOT only has a muscle effect in the context of an existing muscle injury.

These findings differ from those of Körpınar and Uzun.[16] in which three sessions of HBOT at 2.0 and 2.4 ATA promoted oxidative stress in healthy rats, evidenced by higher plasma malondialdehyde and lower plasma superoxide dismutase levels compared to healthy rats that did not receive HBOT. This discrepancy may relate to the variation in HBOT sessions—that is, the healthy rats in the Bosco et al.[13] and Haapaniemi et al.[10] received only one session. Also, the tissue source of measurement (plasma *versus* muscle) may explain the differences as the chemical environments vary among tissues,[17,18] which may influence the effects of HBOT on oxidative stress/antioxidant parameters.[16]

Hyperbaric oxygen therapy on myotoxic-agent-induced skeletal muscle injury

All investigations

In four investigations, a muscle injury was induced by injecting the myotoxic agent—bupivacaine or cardiotoxin—into a target muscle, such as the hindlimb extensor digitorum longus,[19] soleus,[20] or tibialis anterior[21] muscle or face masseter muscle.[22] One or two injections occurred on a single day.[19-22] Three studies involved surgically exposing or making an incision in the targeted muscle to administer the myotoxic agent.[19,20,22] The use of preemptive or postprocedure opioid analgesia was not mentioned in any of the reports. In all studies, only male rats were used (**Table 2**).

The experiments varied in the hyperbaric oxygen regimen (**Table 2**). Although HBOT started the day of or after the injection, the regimen entailed 98.5–100% oxygen and 2.0–3.0 ATA, lasted 60–120 minutes per session, and occurred daily (with or without any breaks) for up to 25 days post-injury.[19-22] For a control comparison, a group of animals received muscle injury and then were exposed to normal room air and atmospheric pressure.[19-22] In addition, Horie et al.[21] allocated one set of injured animals to receive hyperbaria (2.5 ATA) at room air (hyperbaric normoxia) for 2 hours and another set to receive normobaria (1 ATA) and 100% oxygen (normobaric hyperoxia) for 2 hours. Certain data differed among HBOT, hyperbaric air, and normobaric hyperoxia groups; however, no adverse effects associated with HBOT were described in any of the myotoxic agent injury reports, and no experiments involving healthy animals (without muscle injury) were reported in these investigations. These results are described in greater depth in the Discussion section.

HBOT effects in the targeted muscles were measured during early and late periods post-injury. Effects focused on contractile properties and muscle regeneration. **Table 2** lists selected HBOT outcomes. Only one group of researchers did not find an HBOT effect.[22] Horie et al.,[21] who employed a regimen involving 10 days (two sets of 5 consecutive days) of periodic HBOT at 2.5 ATA, continued to observe increased fiber cross-sectional area in the muscle of HBOT rats at 15 days post-injury.

Varying pressure and doses

Two studies were performed that involved testing different pressures or daily doses. After 14 days of HBOT, Gregorevic et al.[19] found that contractile properties and fiber cross-sectional area were greater in rats treated with 3.0 ATA than in 2.0 ATA. In 2002, Gregorevic et al.[20] observed that a 14-day treatment of 3.0 ATA resulted in a higher injured muscle force relative to control muscle force compared to no treatment. However, this effect was not observed at 25 days post-injury.[20] These findings[19,20] indicate that pressure level and/or treatment duration may influence HBOT effects.

Hyperbaric oxygen therapy on crush-induced skeletal muscle injury

All investigations

The six crush investigations involved dropping a mass from a certain distance onto a targeted muscle group, such as the hindlimb muscles, the gastrocnemius[23-27] or tibialis anterior.[28] **Table 3** shows that five studies used rats and included the mass and distance of the model.[23-27] However, one study using mice did not state the mass and distance.[28] The use of preemptive or postprocedure opioid analgesia was not mentioned in any of the reports. Yamamoto et al.[23] used the analgesic agent, felbinac gel, post-injury. In five experiments, the use of males and females was clearly stated.

Five of the six studies tested one or two hyperbaric oxygen regimens. In the remaining study, Yamamoto et al.[26] tested more than two regimens, described in detail below. Among these five studies, HBOT was started within minutes to days post-injury and delivered for 60 or 120 minutes in multiple doses or sessions over days to weeks. As indicated in **Table 3**, the studies' endpoints occurred minutes to days to weeks after the first treatment. These investigations mostly compared the animals in the HBOT group to a no-treatment group. Cervaens et al.[25] also compared HBOT to hyperbaric normoxia and found certain differences between these two groups. These results are described in the Discussion section. Furthermore, no adverse effects associated with HBOT were described in any of the reports.

Table 1 | Overview of HBOT on tourniquet-induced skeletal muscle injury

Study	Year	Animal			HBOT				Outcome[a]
		Species	Sex	Weight (g)	Timing	Pressure (ATA)	Session duration (min)	Sessions	
Nylander et al.[7]	1987	Rat	Male	Not stated	After[b]	2.5	45	1, 2, or 3	↑ATP ↑PCr ↓Lactate (after 3 sessions)
Nylander et al.[8]	1989	Rat	Male	250	After[b]	2.5	45	1	=TBAR at 15 min and 5 h reperfusion ↑TBAR at 45 min reperfusion =TBAR to 3 h of anesthesia only
Sirsjö et al.[9]	1993	Rat	Male	200–250	After[b]	2.5	45	1	↑Functional capillary density leads to ↑blood flow
Haapaniemi et al.[10]	1995	Rat	Male	~250	After[b]	2.2	45	1	↑Glutathione ↑ATP ↑PCr =Lactate
Haapaniemi et al.[11]	1996	Rat	Male	250±20	After[c]	2.2	45	7[d]	↑ATP ↑PCr ↓Lactate
Bosco et al.[13]	2007	Rat	Male	300–350	During[e]	2.8	60	1	=SOD ↓Catalase ↓MDA
Vidigal et al.[12]	2007	Rat	Male	250–300	During[e], after[b], or both	2.0	60 or 120	1	↓Edema ↓Neutrophils ↓Necrosis ↓Red blood cells =Apoptosis
Koca et al.[14f]	2010	Rat	Male	280–340	Before[g]	3.0	60	7[d]	=SOD ↓MDA ↑GSH-Px ↓iNOS staining score/intensity
Frisby et al.[15]	2022	Mouse	Male and female	20–27	Before[h]	2.5	60	1	↓ROS =Leukocytes =Infarct size =Direct muscle- and sciatic nerve-stimulated gastrocnemius maximum tetanic force =ATP

[a]Outcomes reflect one or more endpoints and compared to no treatment (muscle injury without hyperbaric oxygen) unless otherwise stated. [b]HBOT was administered immediately (0 hours) after tourniquet release. [c]HBOT was administered at 0–40 hours after tourniquet release. [d]Sessions occurred over more than one day. [e]HBOT was administered during the final hour of ischemia. [f]In addition to the rubber band, this study involved clamping the common iliac artery via an inguinal incision. [g]HBOT started 72 hours before ischemia. [h]HBOT was administered one hour before tourniquet application. =: No change or difference from no treatment; ↑: increased; ↓: decreased; After: HBOT administered after the completion of ischemia; ATA: atmospheres absolute; ATP: adenosine triphosphate; Before: HBOT administered before ischemia; Both: HBOT administered during and after ischemia; During: HBOT administered during ischemia; GSH-Px: glutathione peroxidase; HBOT: hyperbaric oxygen therapy; iNOS: inducible nitric oxide synthase; MDA: malondialdehyde; PCr: phosphocreatine; ROS: reactive oxygen species; SOD: superoxide dismutase; TBAR: thiobarbituric acid reactive material.

Table 2 | Overview of HBOT on myotoxic agent-induced skeletal muscle injury

Study	Year	Animal			HBOT after injury			Outcome[b]
		Species	Sex	Weight (g)	Pressure (ATA)	Session duration (min)	Sessions[a]	
Gregorevic et al.[19]	2000	Rat	Male	350–400	2.0	90	14 or 25	No effects (absolute values)
					3.0	60	14	↑Specific maximum tetanic force (absolute values)
Gregorevic et al.[20]	2002	Rat	Male	400–450	3.0	60	14 or 25	14 & 25 d: ↑Specific maximum tetanic force (absolute values) =Fiber csa 14 d: ↑% maximum tetanic force (injured/control) 25 d: =% maximum tetanic force (injured/control)
Bajek et al.[22]	2011	Rat	Male	200–250	2.2	60	Up to 10	=MyoD and myogenin protein
Horie et al.[21] (cardiotoxin)	2014	Rat	Male	200–250	2.5	120	10[c]	3 d: ↑$Pax7^+$/$MyoD^+$ nuclei 3 & 5 d: ↑MyoD mRNA 5 & 15 d: ↑Fiber csa 8 d: ↑Tetanus = Fiber csa

[a]The number of sessions equaled the number of treatment days. [b]Compared to the injury + no treatment group. [c]Two sets of 5 consecutive days. ↑: Increased; =: no change or difference from no treatment; ATA: atmospheres absolute; csa: cross-sectional area; HBOT: hyperbaric oxygen therapy.

Among the five studies, the HBOT effects focused on muscle damage or contractile properties, muscle bioenergetics, muscle inflammation and angiogenesis, and muscle regeneration and depended on the specific post-injury timing (**Table 3**). Specific changes in muscle interleukin 6 (increased[27]), phosphorylated total signal transducer and activator of transcription 3 (increased[27]), and vascular endothelial growth factor (VEGF) (increased[23]) protein levels occurred at 3 hours post-injury. HBOT effects on leukocyte infiltration were evident at 1 day post-injury and through 7 days post-injury.[27] Increases in markers of muscle regeneration started at 3 hours post-injury[23] and were evident as late as 4 weeks post-injury[28] (**Table 3**). Certain muscle contractile properties were higher in hyperbaric oxygen–exposed muscle at 3 days post-injury,[24] 7 days post-injury,[23,27] and 4 weeks post-injury.[28] HBOT improved muscle mitochondria bioenergetics after injury.[25] These findings suggest that HBOT effects on crushed muscle start within hours and may continue for days to weeks after the end of HBOT.

Table 3 | Overview of HBOT on crush-induced skeletal muscle injury

		Animal				HBOT after injury and at 2.5 ATA		
Study	Year	Species	Sex	Weight (g)	Drop mass	Session duration (min)	Sessions[b]	Outcome[a]
Cervaens Costa Maia et al.[24]	2013	Rat	Female	200–250	171 g, 102 cm distance	60	3	3 d: ↓CPK ↑Hardness =Maximum elongation
Cervaens et al.[25]	2018	Rat	Female	250–350	171 g, 102 cm distance	60	4	2 d: =Mitochondrial respiratory activity complex I and II =Mitochondrial transmembrane potential complex I and II
Oyaizu et al.[27]	2018	Rat	Male	250–300	640 g, 25 cm distance	120	Up to 5	3 h: ↑IL-6 ↑p/t STAT3 levels 1 & 3 d: ↑$CD68^+$ cells ↑$Pax7^+MyoD^+$ cells 3 & 5 d: ↑$CD206^+$ cells ↑$Pax7^+MyoD^-$ cells ↑$Pax7^-MyoD^+$ cells 5 d: ↓$CD68^+$ cells ↑Regenerating fibers 7 d: ↑Tensile strength ↑$CD163^+$ cells ↓$CD68^+$ cells
Yamamoto et al.[23]	2020	Rat	Male	250–300	640 g, 25 cm distance	120	Up to 5	3 h: ↑VEGF ↑$Pax7^+Ki\text{-}67^+$ cells 5 d: ↑$eMHC^+$ fibers ↑Fiber csa ↑Regenerating fibers 7 d: ↑Tensile strength
Chiu et al.[28]	2020	Mouse	NS	21–26	NS	120	7 or 14	4 wk after the 7- & 14-d sessions: ↑Regenerating fibers ↑4 wk after the 7-d session: =Fast-twitch strength =Tetanus strength 4 wk after the 14-d session: ↑Fast-twitch strength ↑Tetanus strength
Yamamoto et al.[26]	2021	Rat	Male	250–300	640 g, 25 cm distance	120	Multiple	See text

[a]Compared to the injury + no treatment group. [b]The number of sessions equaled the number of treatment days except for Cervaens et al. [25], which involved four sessions over 2 days. =: No change or difference from no treatment; ↑: increased; ↓: decreased; ATA: atmospheres absolute; CPK: creatine phosphokinase; csa: cross-sectional area; eMHC: embryonic myosin heavy chain; HBOT: hyperbaric oxygen therapy; IL-6: interleukin 6; NS: not stated; p/t STAT3: ratio of phosphorylated signal transducer and activator of transcription 3 to total signal transducer and activator of transcription 3; VEGF: vascular endothelial growth factor.

Muscle oxygen concentration

As part of their study, Oyaizu et al.[27] measured gastrocnemius muscle oxygen concentration before injury and after injury (before HBOT and during and after HBOT) and found that oxygen concentration dropped from 45 to 15 mmHg by 30 minutes of injury and then returned to ~45 mmHg by 3 hours post-injury in the HBOT group. In comparison, the oxygen concentration of the untreated group did not return to the preinjury level until 30 hours post-injury.[27]

Hyperbaric oxygen therapy combined with reactive oxygen species and nitric oxide inhibitors on skeletal muscle injury

Besides investigating the effects of HBOT on muscle angiogenesis, regeneration, and strength, Yamamoto et al.[23] performed inhibitor experiments with N-acetyl cysteine and N-nitro-L-arginine methyl ester hydrochloride to determine whether the HBOT effects on angiogenesis (e.g., angiogenic factors and blood vessel number), muscle regeneration (e.g., regenerating fibers and mean fiber cross-sectional area), and muscle strength were regulated by reactive oxygen species (ROS) and nitric oxide (measured as nitrite). Yamamoto et al.[23] noted that N-acetyl cysteine is part of the glutathione production pathway and contains a thiol group, which can inhibit all ROS. The HBOT started immediately after the injury and continued for up to four more days post-injury.[23] The inhibitors were administered peritoneally; two doses were administered prior to the injury; and post-injury doses were injected 30 minutes before HBOT and for 2 days after the cessation of HBOT.[23] The timing of measures varied depending on the outcome, including before injury and from 3 hours post-injury to 7 days post-injury.[23] For muscle nitrite, HIF1α and basic fibroblast growth factor (3 and 6 hours post-injury) levels, immature blood vessel number, and muscle regeneration and strength (twitch force), the inhibitors yielded similar effects, whereas N-acetyl cysteine reduced muscle VEGF levels more than N-nitro-L-arginine methyl ester hydrochloride at 3 and 6 hours post-injury, and only N-nitro-L-arginine methyl ester hydrochloride decreased mature blood vessel number at 3 days post-injury.[23] They concluded that HBOT effects on muscle recovery may be mediated by nitric oxide. However, in this context, it is unclear whether nitric oxide is acting as a reactive species or antioxidant. Yamamoto et al.[23] also did not evaluate the concentration of antioxidants.

These inhibitor experiments indicate that ROS, nitric oxide, HIF1α, and basic fibroblast growth factor are involved in HBOT effects after crush muscle injury. In various articles, the role and targets of ROS in signaling pathways have been reviewed in the context of hyperbaric oxygen,[29,30] skeletal muscle regeneration,[31] and in general.[32] Although none of these reviews address crush injuries, the findings[23] align with these reviews.

Varying conditions of hyperbaric oxygen therapy on skeletal muscle injury

In another experiment, different groups of varying session number (1, 3, or 5 sessions) and initial timing of HBOT (0, 1, 3, or 5 days post-injury) were compared to a no-treatment group regarding three major outcomes (i.e., muscle regeneration, muscle strength, and macrophage number)[26]; the outcomes measured at one or more endpoints consisted of one preinjury evaluation and up to four post-injury time points (1, 3, 5, and 7 days post-injury).[26] The top three hyperbaric oxygen regimens that yielded positive changes in the major outcomes were regimens of five sessions (initiated immediately after injury) and three sessions (initiated soon after injury and 1 day post-injury).[26] Therefore, HBOT effectiveness may be influenced by multiple sessions started during the early phase of regeneration.

At 4 weeks post-injury, Chiu et al.[28] compared the number of regenerating myofibers (myofibers with centrally located nuclei) and muscle strength of mice that received seven HBOT sessions to those that received 14 HBOT sessions. While both regimens increased the number of regenerating myofibers, this count and muscle strength were higher in the mice that received 14 sessions than in those that received seven sessions.[28] Both doses indicate a 2–3-week sustained HBOT effect relating to the number of regenerating myofibers.

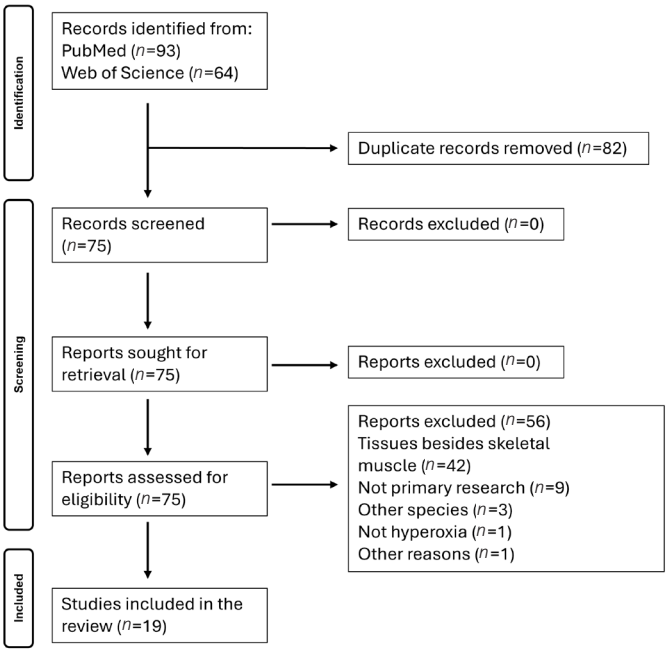

Figure 1 | Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 flow diagram. This flow diagram[6] shows the data selection process of records. The top right box was shortened from the original. This diagram is licensed under Creative Commons Attribution (CC BY 4.0), https://creativecommons.org/licenses/by/4.0/.

Discussion

Collectively, the data indicate that HBOT affects multiple responses to three major types of acute muscle injury: tourniquet, myotoxic agent exposure, and crush. However, studies focused on myotoxic agents and crush injury have generated more evidence related to muscle regeneration than tourniquet investigations. Moreover, this evidence shows that HBOT positively affects muscle regeneration. There is a need to determine whether tourniquet-HBOT treatment investigations lead to similar positive outcomes as many clinical conditions can involve prolonged muscle ischemia-reperfusion.

Evaluation of preclinical hyperbaric oxygen therapy and muscle injury research and recommendations for future research

The adoption and standard use of HBOT as a clinical treatment for limb trauma and muscle regeneration could depend partly on the scientific foundation generated by preclinical research. As such, the evaluation of the methods of these investigations can lead to suggestions for advancing the rigor and translation of preclinical research by (a) recognizing the preclinical HBOT characteristics that overlap with the clinical HBOT characteristics, (b) identifying the gaps in HBOT preclinical research from a clinical perspective, and (c) describing the discrepancies between preclinical research and clinical practice. This evaluation will be performed by focusing on three categories: parameters specific to HBOT (pressure levels, sessions, and timing), clinical attributes (biological sex and preemptive and/or postprocedure opioid analgesia use), and research design (control or comparison groups and experimental measurement interval). **Figure 2** summarizes these categories as considerations for future preclinical HBOT research.

Pressure

All preclinical muscle studies used consistent pressure as part of HBOT. Across the experiments, hyperbaria ranged from 2.0 to 3.0 ATA, focusing on more moderate (2.0–2.4 ATA) and higher (> 2.4 ATA) levels. The milder level, 1.1–1.9 ATA (with 100% oxygen), remains untested. Both tourniquet and myotoxic agent studies covered two pressure levels. In contrast, the crush studies only covered the higher level. Hadanny and Efrati[29] indicate that current HBOT protocols (in the clinical setting) do not surpass 2.4 ATA (to avoid adverse pulmonary and central nervous system effects), so many tourniquet and myotoxic agent studies use a clinically relevant pressure. Interestingly, no adverse effects were reported in the preclinical studies that used > 2.4 ATA. Rodents may be less prone to these adverse pulmonary and central nervous system effects.

Recent findings indicate that the pressure level may lead to differential mechanistic effects. In a non-muscle injury study involving healthy human females (n = 2) and males (n = 12), Leveque et al.[33] investigated blood or urinary reactive species (i.e., ROS production, nitric oxide, isoprostane), antioxidant (i.e., catalase, cysteinylglycine, glutathione, and superoxide dismutase), and inflammatory (i.e., interleukin 6, neopterin, and creatinine) kinetics after 1 hour of exposure to milder (1.4 ATA with 100% oxygen) and higher (2.5 ATA with 100% oxygen) pressure levels. One major finding was that ROS and antioxidant kinetics were similar between the two pressure levels.[33] In contrast, neopterin and creatinine increased more after exposure to 2.5 ATA than 1.4 ATA.[33] Therefore, in the context of tissue or muscle regeneration, the pressure level of HBOT may need to be considered with the time course or status of the regeneration. For example, immediately after injury, a milder pressure level may be appropriate to activate ROS and antioxidants. This milder pressure level may widen the access of HBOT if portable or smaller chambers can provide this pressure level. As stated earlier, access to HBOT is limited because of the required specialized equipment and staff.[2]

Another consideration related to pressure level is adverse effects. Although no adverse effects associated with HBOT were reported in these rodent muscle injury studies, adverse effects have been reported in studies involving patients. A systematic review and meta-analysis of children and adults mostly undergoing HBOT for extremity ulcers, stroke, traumatic brain injury, and other neurological conditions revealed the occurrence of 11 categories of adverse effects.[34] However, only two of these effects, ear discomfort and visual changes, displayed greater risk ratios in patients receiving HBOT in contrast to those receiving sham or conventional therapy (control patients).[34] Moreover, Zhang et al.[34] reported that adverse effects were (a) higher in patients receiving ≥ 2.0 ATA than control patients and (b) similar between patients receiving < 2.0 ATA and control patients.

Collectively, these results and practices support testing milder and more moderate pressure levels, < 2.5 ATA in preclinical studies, especially those involving crush injury. In addition, more comparative studies of different pressure levels would expand our understanding of the positive effects most dependent on milder, moderate, and higher pressure levels. Through this knowledge, exposure to higher pressure levels could be limited.

Sessions

As mentioned earlier, HBOT is typically administered once per day for a certain number of days or sessions.[35] However, Balestra et al.[35] suggest that the scientific foundation of HBOT

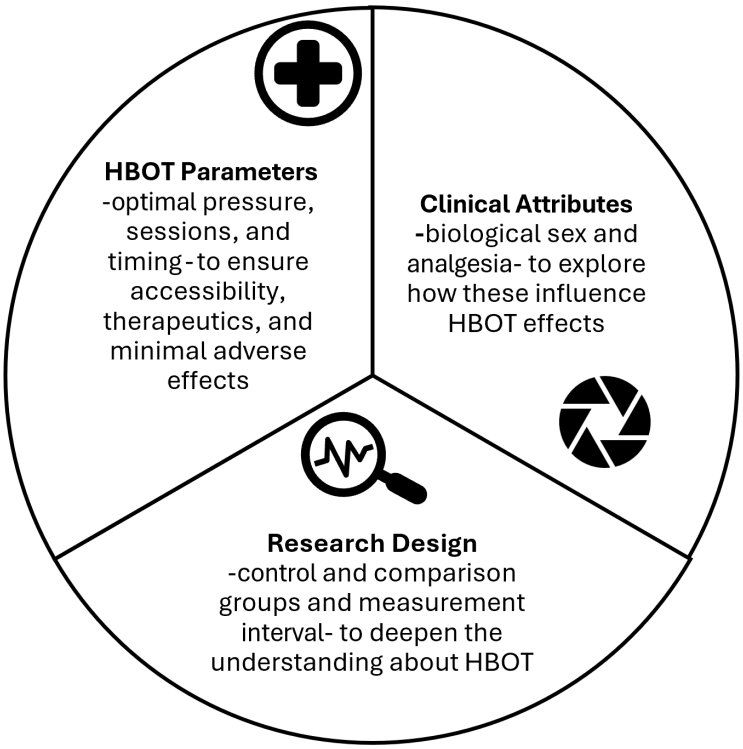

Figure 2 | **Considerations for future preclinical HBOT research.**
HBOT parameters, clinical attributes, and research design represent three preclinical research areas for advancing the understanding of the effects of HBOT on muscle injury. This diagram was created using Microsoft PowerPoint software build 16.0.18725.40515. HBOT: Hyperbaric oxygen therapy.

may benefit from systematic optimization of session number and frequency. Among the HBOT characteristics of the preclinical muscle studies, the total session number varied, ranging from 1 to 25, and most sessions occurred daily (**Tables 1–3**). The tourniquet studies usually involved one session, whereas the myotoxic and crush studies involved multiple daily sessions.

Recently, Schottlender et al.[30] reviewed the conditions under which HBOT would lead to differential effects involving mitochondrial activity, ROS production, and antioxidant levels. Primarily, fewer than six consecutive HBOT sessions will initiate increased ROS production, whereas 20–60 consecutive sessions increase mitochondrial activity and may achieve sustained, elevated antioxidant levels or activity.[30] According to Schottlender et al.[30] ROS and reactive nitrogen species production from early HBOT sessions are important for directing cellular healing and survival mechanisms.

Körpınar and Uzun[16] also published findings supporting the session number effects. At 2.0 and 2.4 ATA, 15 HBOT sessions over 10 days administered to healthy rats resulted in lower plasma levels of malondialdehyde (oxidative stress) and higher plasma levels of superoxide dismutase (antioxidant parameter); however, only 2.4 ATA led to higher erythrocyte glutathione levels with more sessions.[16] These findings indicate that a greater number of sessions upregulates antioxidant protection.

As mentioned earlier, Yamamoto et al.[23] observed that HBOT led to increased VEGF and HIF1α in crush-injured muscle. ROS and nitric oxide inhibitors blocked these increases, suggesting a role in HBOT. Therefore, the early positive effects of HBOT for acute muscle injury, as described above, may be through oxidative stress. Antioxidants may be responsible for the later positive effects.

Another consideration related to the total session number is the occurrence of adverse effects. As mentioned, no adverse effects were reported in the preclinical muscle injury studies. However, in their systematic review/meta-analysis, Zhang et al.[34] found that patients who received more than 10 HBOT sessions reported a higher number of adverse effects than those patients who did not receive HBOT. These data suggest that HBOT session optimization may establish the requisite total session number to elicit the most favorable cellular responses.[35] Also, session optimization may aid in reducing patient exposure to HBOT adverse effects.

Timing

Generally, HBOT was administered after the muscle injury, which simulates the clinical situation. However, one consideration is the specific timing after injury. As described, Yamamoto et al.[26] investigated different days after injury to initiate HBOT and found that HBOT was most effective when initiated no later than 1 day post-injury. Clinical data indicate that introducing HBOT within 1 day post-injury may be salient to avoiding complications of crush injury. For example, several patients who experienced subacute/late complications after an upper limb crush injury and surgery received HBOT after the first 24 hours post-injury.[3] Preclinical studies could be standardized and more clinically relevant by introducing HBOT within 1 day post-injury.

Biological sex

In general, these HBOT and muscle investigations have involved male rats and mice. Only one tourniquet study[15] and two crush studies included females.[24,25] While Hart and Strauss[36] reported gender differences in oxygen uptake by muscle, the reason for this difference remains unclear. In addition, the elements of muscle regeneration can vary between males and females,[37,38] and estrogen has specific effects on muscle regeneration.[39,40] Additionally, the source of muscle-derived stem cells (MDSCs) affects muscle regeneration. Deasy et al.[41] found that MDSCs from female mice exhibited a higher regeneration index and regenerated a greater number of myofibers than MDSCs from male mice. However, stimulation of the male MDSCs with 17-β estradiol failed to increase regeneration efficiency.[41] Collectively, these findings indicate that more studies with female rats or mice are needed to understand whether biological sex moderates the effects of HBOT on muscle regeneration or whether the previous effects observed in males are reproducible in females.

Use of preemptive and postprocedure opioid analgesia

In the reviewed preclinical muscle injury studies, none of the investigators report administering opioid analgesia to the rats and mice. This lack of reporting could mean that the animals were not treated with either preemptive or postprocedure opioid analgesia. In contrast, patients with trauma often receive opioid analgesia.[42,43] Since the animals' analgesic or pain/distress status may differ from patients treated with HBOT, the question arises whether, in a preclinical acute muscle trauma context, the presence of opioids influences HBOT's physiological mechanisms and, ultimately, muscle outcomes. While no data were found to answer this question, short-term opioid exposure can increase antioxidants[44,45] and mitigate oxidative stress.[45] HBOT activates both oxidative stress and antioxidant activity,[30] so the presence of opioids could influence HBOT's effects. Muscle outcomes as a response to HBOT may vary depending upon the concomitant absence/presence of opioid exposure. Therefore, preclinical muscle injury studies are needed that use opioids during the administration of HBOT.

Control or comparison groups

In most of these muscle injury studies, researchers compared HBOT to no treatment—that is, animals exposed to normobaria and normoxia to address experimental validity. However, since HBOT represents a combination of hyperoxia and hyperbaria, this comparison does not address the specific contributions of hyperbaric air alone (pressurized air at room air [normal oxygen level]) or normobaric hyperoxia (100% oxygen at room atmospheric pressure) to the outcomes. Only one myotoxic agent[21] and one crush[25] investigation compared the HBOT group to a normobaric hyperoxia and/or hyperbaric air groups. The effects of these single-condition groups were not always

the same as those of the no-treatment or HBOT groups.

Hyperbaric air effects

In one crush study, higher mitochondrial respiratory activity complex I parameters were detected in the hyperbaric air group compared to the no-treatment and HBOT groups.[25] In addition, Cervaens et al.[25] observed differences in mitochondrial transmembrane electric potential between hyperbaric air and no-treatment groups and HBOT groups. Furthermore, HBOT-induced changes in mitochondrial transmembrane electric potential were observed for complex I parameters compared to hyperbaric air, but HBOT and hyperbaric air differences were not observed for complex II parameters.[25] To date, only Fujita et al.[46] tested the effect of hyperbaric air on muscle regeneration (after intramuscular bupivacaine injection) by treating male rats to 1.25 ATA continuously (except for cage cleaning breaks) for up to 28 days and comparing these rats to a no-treatment group. They reported these results in the hyperbaric air group: at 1 day post-injury, increased $CD68^+$ staining, increased $CD68^+CD206^+$ staining, and increased tumor necrosis factor alpha (*Tnfα*) and interleukin 10 (*Il10*) mRNA levels; 5 and 7 days post-injury, larger fiber cross-sectional area, decreased *Il10* mRNA levels, and higher count of $Pax7^+MyoD^+$ nuclei; 7 days post-injury, decreased $CD68^+$ staining; and 14 and 28 days post-injury, no change in fiber cross-sectional area.[46] While some of these findings overlapped with Oyaizu et al.[27] as listed in **Table 3**, the delivery of continuous hyperbaric air neither simulates the intermittent approach nor the clinical administration of HBOT.

Normobaric hyperoxia effects

As part of their cardiotoxin-muscle injury investigation, Horie et al.[21] reported a lower regenerating fiber cross-sectional area in the normobaric hyperoxia group than that of the HBOT and hyperbaric air groups at 5 days post-injury. However, at this same day post-injury, the regenerating fiber cross-sectional area was higher in both the HBOT and hyperbaric air groups compared to the no-treatment group.[21] These investigators also found that at 5 days post-injury, myogenin mRNA levels were both lower in the normobaric hyperoxia and hyperbaric air groups compared to the HBOT group, but these levels were higher in the normobaric hyperoxia group than the hyperbaric air group.[21]

Besides these muscle injury studies, other healthy muscle and *in vitro* investigations utilizing C2C12 muscle cells have been conducted to understand the effects of normobaric hyperoxia. At normobaria, Flandin et al.[47] exposed live male C57BL/6 mice with uninjured muscle to room air or 100% O_2 for 3 days and C2C12 myotubes to 95% O_2/5% CO_2 for 2 days. The hindlimb muscle of the live mice and C2C12 myotubes demonstrated oxidative stress.[47] More recently, Horiike et al.[48] exposed C2C12 cells to 95% air/5% CO_2 or 95% O_2/5% CO_2 from 2 hours to 24 hours at normobaria and then evaluated cellular proliferation and other activities. A key finding is that normobaric hyperoxia exposure for 2 and 4 hours elicited positive cellular effects (e.g., increased proliferation) in the presence of the antioxidant vitamin C compared to normal air exposure with this vitamin.[48]

Collectively, the results indicate that a comparison of an HBOT group to a no-treatment group will demonstrate what to expect under both normobaria and normoxia. Including normobaric hyperoxia and hyperbaric air groups as comparison groups may also indicate the specific contribution of increased pressure, oxygen, or both to certain effects. Based on the varied *in vivo* findings comparing HBOT to hyperbaric air or normobaric hyperoxia, future HBOT preclinical research focused on muscle regeneration could incorporate these additional comparison groups, strengthening experimental validity. In addition, for certain effects, hyperbaric air or normobaric hyperoxia may be sufficient for clinical treatment, which may be more cost-saving and acceptable to patients.

Measurement interval

In most experiments, effects were measured following the initiation of, during, or immediately after HBOT. However, both Horie et al.[21] and Oyaizu et al.[27] continued to observe positive effects a few days after HBOT ended (**Tables 2** and **3**). As described above, Chiu et al.[28] observed sustained effects weeks after treatment. These findings raise the point of whether HBOT has sustained effects.

The data also suggest that longer HBOT does not consistently result in more or better outcomes. For example, Gregorevic et al.[20] treated mice that had undergone myotoxic agent injury of the soleus muscle for 14 days with HBOT (3.0 ATA) and observed a positive contractile property outcome after HBOT compared to no treatment. This effect was absent in mice treated for 25 days with HBOT.[20] Furthermore, in the myotoxic-agent-injured extensor digitorum longus muscle, neither 14 or 25 days of HBOT (at 2.0 ATA) generated a positive contractile property (absolute values).[19] In addition, there was no difference in fiber cross-sectional area, an indication of regenerating fiber maturity, at 14 and 25 days post-injury compared with no treatment.[20]

However, longer HBOT yields different results than a shorter regimen after muscle injury. Gregorevic et al.[19] found that 2.0 ATA HBOT for 25 days resulted in a relative cross-sectional area of regenerating extensor digitorum longus myofibers almost one-third greater than in a no-treatment group. Also, with the same treatment and no-treatment groups, these researchers reported a greater maximum isometric tetanic force with 25 days of 2.0 ATA.[19] These two positive effects were not observed with 2.0 ATA for 14 days.[19] Chiu et al.[28] found that a longer HBOT time (14 days post-injury) after injury of the tibialis anterior muscle yielded greater muscle function and number of regenerating myofibers than a shorter treatment time (7 days post-injury).

These findings suggest that the specific effect of interest, the targeted muscle, and/or the type of muscle injury may influence the total HBOT session number. Sustained effects could result in overall shorter HBOT, improved adherence, and reduced costs. Therefore, more preclinical investigations are needed to examine the effects of HBOT from days to weeks after HBOT cessation.

Limitations

There are two major limitations of this review. First, only 4–6 studies were reviewed for the myotoxic agent and crush injuries. Second, with the limited number of studies, biases and quality of individual studies were not systematically assessed.

Conclusions

We conducted a scoping review of the preclinical muscle injury research involving HBOT to determine the current state of the science. Key findings are that HBOT positively affects muscle antioxidant activity and many indicators of muscle regeneration. These preclinical data indicate these effects may be elicited in response to varying pressure levels and session numbers, and these effects are usually elicited when HBOT is initiated soon after injury. However, only one preclinical comparative timing (initiation) study has been conducted.[26] Also, there may be sustained effects, lasting days to weeks after HBOT cessation.

Our evaluation of the state of the science revealed four exciting directions for future research. For example, the inclusion of both males and females in HBOT-muscle regeneration research will broaden our understanding of whether HBOT influences the sex differences in muscle regeneration or whether sex influences the HBOT response. Another recommendation is to test HBOT at milder pressure levels in the crush injury models. If milder pressure levels can be used, then the findings may be more easily translated to the clinical setting. Patients may be more receptive to HBOT at lower pressure levels. Studies focused on identifying specific pathways involved in the HBOT-muscle regeneration response would strengthen the scientific foundation of HBOT. To date, the nuclear factor erythroid 2-related factor-2 pathway, which interacts with ROS,[32] has yet to be examined in any of the preclinical muscle injury models. Another interesting focus would be to determine how opioid analgesia—a critical intervention of injury—interplays with HBOT after muscle injury, as this information would inform effective care planning.

HBOT continues to hold promise as an effective treatment for limb trauma/crush injury. However, there are reservations with conducting HBOT patient research that includes both treatment and control groups. These reservations relate to using a sparse treatment resource for research instead of for emergency or standard treatment or acquiring the attitude that placement inside the chamber without increased pressure or oxygen represents no treatment or withholding an accepted treatment.[5] With these reservations, preclinical research can serve a significant role in the scientific foundation of HBOT.

Acknowledgements: *The authors thank Sarah Lyons, Slash & Dash Ink, LLC for editing assistance.*

Author Contributions: *BSPS conceived of the review and drafted the manuscript. JRW, LZ, and TD also assisted with drafting the manuscript. All authors read and approved the final version of the manuscript.*

Conflicts of interest: *None.*

Declaration of AI and AI-assisted technologies in the writing process: *The authors declare that no Generative AI was used in the preparation of this manuscript.*

Data availability statement: *Not applicable.*

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