REVIEW

Rationale for hyperbaric oxygen therapy in traumatic injury and wound care in small animal veterinary practice

D. M. Levitan [1,*], M. Hitt[†], D. R. Geiser[‡] and R. Lyman[§]

*College of Veterinary Medicine, Long Island University, Brookville, NY, 11548, USA
[†]Atlantic Veterinary Internal Medicine and Oncology, Annapolis, MD, 21401, USA
[‡]College of Veterinary Medicine, University of Tennessee, Knoxville, TN, 37996, USA
[§]Animal Emergency and Referral Center, Fort Pierce, FL, 34982, USA

[1]Corresponding author email: diane.levitan@liu.edu

Hyperbaric oxygen therapy is in wide use in human medicine around the world. Although hyperbaric oxygen therapy is available for veterinary use, it is still significantly underutilised. The physical principles, gas laws and physiologic mechanisms by which hyperbaric oxygen therapy is therapeutic, especially in traumatic injuries and complicated wound care, are discussed. Then, considerations are offered for the implementation of hyperbaric oxygen therapy in veterinary practices. Finally, a review of clinical indications for veterinary practices, including a presentation of select literature, is provided. Applying hyperbaric oxygen therapy in an earlier and more consistent manner could improve short- and long-term outcomes in complicated wounds. The authors also hope this information may stimulate interest in the design of future, prospective studies for the various clinical situations described.

Journal of Small Animal Practice (2021) **62**, 719–729
DOI: 10.1111/jsap.13356

Accepted: 18 April 2021; Published online: 21 May 2021

INTRODUCTION

In the past several decades, the field of veterinary trauma and wound care has progressed and mirrored that of human care. The use of hyperbaric oxygen therapy (HBOT) has grown in both human and veterinary trauma facilities. In veterinary and human medicine, HBOT is frequently employed in the treatment of necrotizing fasciitis, crush injuries, infected and complicated wounds, and burns (Goldstein *et al.* 2006, Latimer *et al.* 2018). HBOT in human medicine is also commonly used for diabetic non-healing wound care, delayed radiation injuries, ischemia–reperfusion injuries and disorders, infected abscesses, complicated grafts and flaps, and other medical conditions (Goldstein *et al.* 2006, Zhang *et al.* 2008, Thom 2009, Thom 2011, Thom 2012, Weaver 2014).

HBOT is in wide use in human medicine around the world. While the Undersea and Hyperbaric Medical Society (UHMS), a USA-based organisation for human medicine, has 14 indications, which are approved and thus reimbursed by third party payers, Japan has 20 approved indications, Russia has 24 and China has 60 (Jain 2017). Although HBOT is available for veterinary use, it is still significantly underutilised, in part due to lack of familiarity with the therapeutic approach, a subjectively high initial investment in facilities and team training, and in part due to lack of compelling data from well-designed studies in the veterinary peer-reviewed literature. However, most veterinary pet insurance companies cover HBOT, as long as the therapy is prescribed by a licensed veterinarian.

Traumas, especially blunt and/or penetrating wounds, are a common presentation to veterinary practices, accounting for up to 30% of emergency visits to primary care veterinary clinics (Saito & Rhoads 2003). Wound care in particular is a common presentation to small animal practices, with bite wounds, dehiscence of old wounds and laceration identified as the most common wound presentations (Kožár *et al.* 2018). This article is a discussion of the basis for the use of HBOT in traumatic injuries and wound care, and an overview of some of the preliminary work done to translate this emerging therapeutic into an established practice for veterinary medicine.

HBOT has been explored as a therapy since 1960 (Boerema 1959). HBOT is a treatment in which a patient breathes 100% oxygen while inside a pressurised chamber. To standardise units of pressure, atmospheres absolute (ATA) is used to describe the treatment pressure. ATA includes the inherent 1 atm pressure at sea level, as well as

the additional pressure developed inside the chamber. Increasing the pressure of the environment, including the pressure of inhaled gas, causes the amount of dissolved oxygen in the blood to increase.

The use of HBOT for wound care is not meant for healthy, normal tissues. Although it may be helpful in many ways for generalised healing, it is best applied in situations where healing is impaired, such as with severe swelling, infection, crush injury, radiation scars and hypoxic tissue. Hyperbaric oxygen is meant to be used as an adjunct to the management of issues where there is chronic oxygen deficiency and the local oxygen tension is not optimal for healing. Studies that compare normal healthy tissue healing times to time to healing with the use of hyperbaric oxygen should not expect to find significant healing differences. Tissue that is unhealthy, however, with poor oxygen tensions for any reason is expected to heal better with adjunctive HBOT (Niinikoski 2004).

GAS LAWS AND THE SCIENTIFIC BASIS FOR HBOT

In a famous "Life Without Blood" experiment in 1960, Dr. Ite Boerema demonstrated that hyperbaric oxygen alone kept exsanguinated pigs alive in the absence of haemoglobin (Boerema 1959). This experiment was foundational as early evidence that physics properties were applicable *in vivo* with HBOT. The mechanism of action for this therapy is founded in a number of gas laws, summarised in Fig. 1 and as follows.

Boyle's law

Boyle's law states that the volume of a gas is inversely related to the pressure and the density is directly related to the pressure (Hardy *et al.* 2008). Thus, under an increase in pressure, the volume of a gas will decrease and the density of the gas will increase. As the volume of gas decreases, the oxygen molecules in the alveolus become more concentrated.

Dalton's law

Dalton's law states that in a mixture of non-reacting gases, the total pressure exerted is equal to the sum of the partial pressures of the individual gases (Hardy *et al.* 2008). In room air, oxygen is 21% of the gas mixture, which equals 160 mmHg of pressure, 21% of the total 760 mmHg at 1 atm. The rest of the partial pressures are made from nitrogen (78% or 590 mmHg), argon and other gasses (1% or ~ 8 mmHg).

Breathing 100% oxygen means that the *total* gas pressure is exerted by the oxygen alone. At sea level, this pressure is equal to 760 mmHg. This is a significant increase compared to air, which is 160 mmHg of pressure from oxygen.

Graham's law

Graham's law describes the diffusion of gases from high pressure to low pressure (Hardy *et al.* 2008). During HBOT, the patient breathes 100% oxygen *and* is breathing oxygen at a significantly higher pressure. For example, during a treatment at 2 ATA, the pressure from the 100% oxygen gas being inspired is doubled, from 760 to 1520 mmHg.

Breathing pure oxygen at 2 ATA results in over nine times the oxygen pressure when compared to breathing ambient air at sea level. This provokes an increased diffusion of oxygen into the blood. When the circulating plasma-dissolved oxygen reaches the tissues, this increased concentration yields increased diffusion of oxygen into the tissue.

Henry's law

Henry's law describes the behaviour of gases when they come into contact with a liquid, such as blood (Hardy *et al.* 2008). Henry's

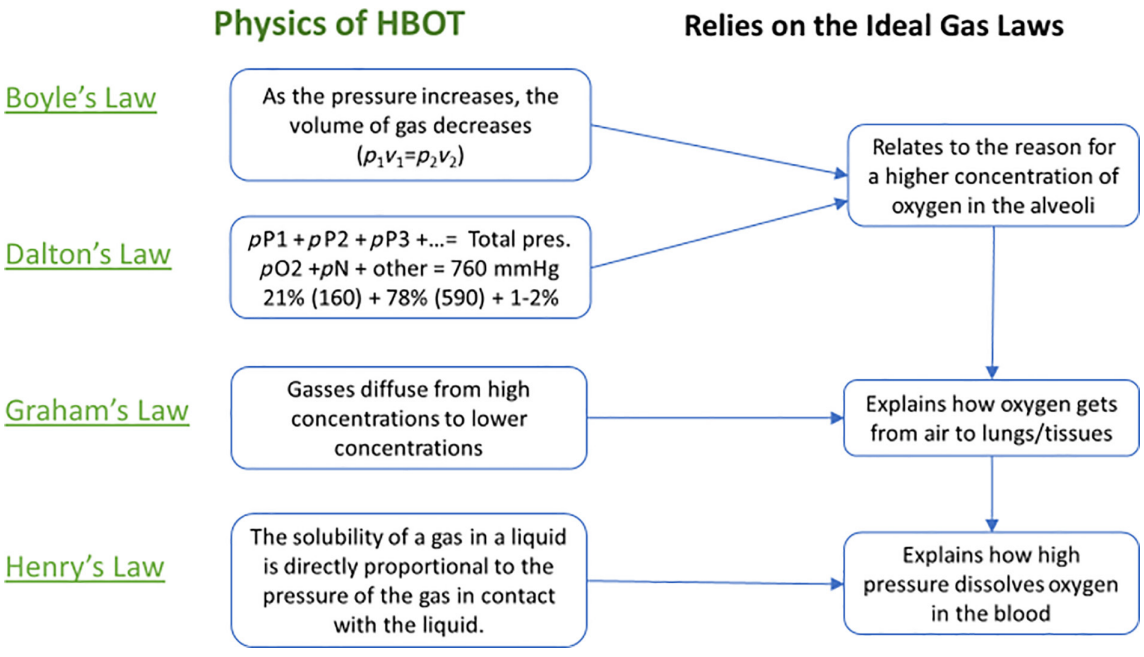

FIG 1. Gas law summary

law states that the concentration of gas in a liquid is directly proportional to the solubility and partial pressure of that gas. The greater the partial pressure of the gas, the greater the number of gas molecules that will dissolve in the liquid. According to Henry's law, oxygen is more soluble in blood when under pressure.

The increased oxygen concentration in pressurised air results in more oxygen molecules at the interface of the alveoli and the blood. As a result, more oxygen diffuses from high concentration to lower concentration, and therefore, more oxygen molecules diffuse into the arterial blood, as depicted in Fig. 2.

PHYSIOLOGIC MECHANISMS

Wound healing is the process of the body to replace damaged tissue with living tissue. Wound healing mechanisms have been studied extensively. The stages of wound healing have been established to be overlapping phases that are inflammation, proliferation and remodelling. If there is any disruption in these phases, there will be abnormal wound healing (Wernick & Stawicki 2020).

HBOT plays a key role when there is abnormal wound healing. There are multiple overlapping and intertwined mechanisms by which this can occur.

Vasoconstriction and reduced inflammation

Excessive inflammation is a source of prolonged tissue repair and poor wound healing (Eming *et al.* 2007). It is also an important contributor to pain. Blood and lymphatic vessel disruption, dilation and widening following damage to tissue result in decreased blood and lymphatic flow. Increase in blood flow from damaged tissue (vasogenic) coupled with increased vascular permeability from cellular injury (cytogenic) leads to tissue swelling/edema (Scallan & Korthuis 2010). This creates increased local pressure, further compromising blood flow. If this pressure approaches or exceeds that in the blood vessels, blood flow will slow or stop altogether. Swelling contributes to tissue hypoxia by increasing capillary distances within tissues.

HBOT initiates generalised vasoconstriction of healthy blood vessels supplying damaged tissues. The hyperoxic environment leads to increased oxidation of nitric oxide (NO) radicals produced by the endothelium, thus leading to a loss of the vasorelaxant effect (Mihaljević *et al.* 2018). Additional research has shown that HBOT leads to alterations in other vasodilator compounds, such as prostaglandins, which contribute to the net vasoconstriction effect. This arteriolar vasoconstriction leads to an overall decrease in tissue edema and increased tissue oxygenation. Improved intracellular oxygen tension maintains mitochondrial and other cellular metabolic functions, which in turn helps maintain integrity of cell membranes and energy-dependent mechanisms. With HBOT, there is a reduction of the vasogenic and cellular fluid shifts that normally result in edema such as seen in crush injuries, compartmental syndromes, burns, reperfusion injury and reimplantation situations (Dünnwald *et al.* 2018, Gasier *et al.* 2018, Mihaljević *et al.* 2018, Robins & Wyatt 2020). The benefits are the sum effects of vasoconstriction and subsequent tissue increase of NO. This decreases tissue edema and improves oxygenation, decreases inflammation and cause for further damage, reduces neutrophil adhesion, and induction of various fibroblasts, and growth factors. The effects of HBOT may not solely be due to vasoconstriction, but could also be due to the transient impairment of vasorelaxation, which recovers 24 hours post-treatment (Mihaljević *et al.* 2018). Any initial negative effects of vasoconstriction to damaged tissues, partly contributed to by reduction in NO, are overcome by the positive aspects of increased oxygenation.

During the inflammatory process post-injury, cytokines and chemokines are released to regulate the body's response to the insult. Chronic inflammation, however, may ensue, which leads to non-healing wounds and scar tissue formation, among other complications. Interruptions to the pro-inflammatory cytokine response show promise in addressing complicated and non-heal-

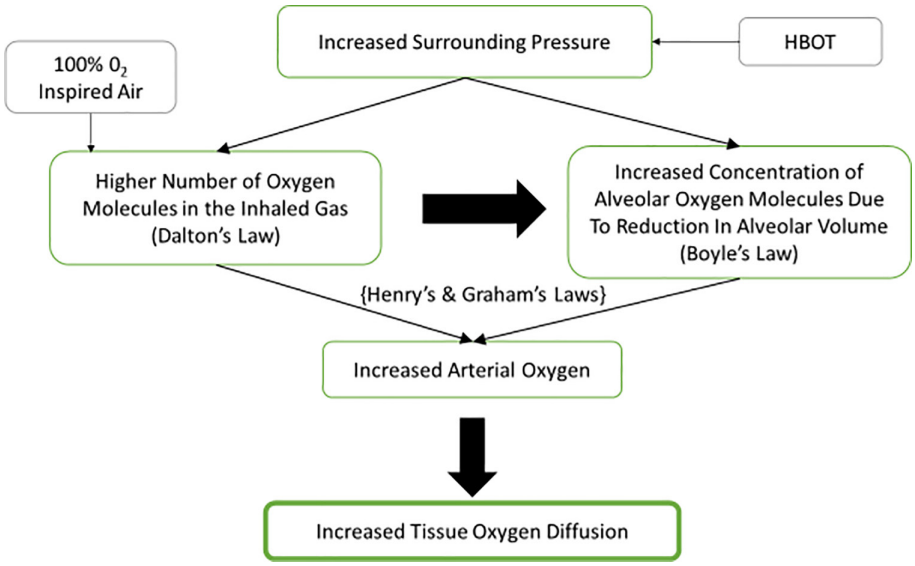

FIG 2. Gas law implications in Hyperbaric oxygen therapy (HBOT)

ing wounds. *In vivo* and *in vitro* studies show that patients with an elevated inflammatory response who undergo successive HBOT sessions will yield reduced proinflammatory cytokine levels, as compared to pre-treatment (Weisz *et al*. 1997, Benson *et al*. 2003, Thom 2012, Benkő *et al*. 2019). In a rat skeletal muscle injury model, 2.5 atm absolute for 2 hours/day after injury demonstrated reduced early lower limb volume and muscle wet weight in contused muscles and promoted muscle isometric strength a week after injury. HBOT suppressed the elevation of circulating macrophages in the acute phase and then accelerated macrophage invasion into the contused muscle thereafter. The number of proliferating and differentiating satellite cells and the amount of regenerated muscle fibres also increased, suggesting decreased inflammation and improved healing (Oyaizu *et al*. 2018).

Another consequence of the excessive inflammatory response is the adhesion of white blood cells (leukocytes) to the vasculature. This adhesion causes injury to the microvasculature, including the degradation of the basement membrane and cytotoxicity to the cells of the vessel wall. This ultimately leads to leakage of fluid out of the vasculature (edema) and reduced protection of the vessel walls (Granger 2010). The influence of HBOT on a hyperinflammatory response is multifactorial, including reducing levels of inflammatory cytokines and reducing cellular vasculature wall adhesions and resultant implications of leaky vasculature. HBOT decreases inflammation by decreasing inflammatory cytokines that result in the cascade of neutrophil rolling, activation, adhesion and transmigration into tissues by impairing sythesis of Cyclic guanosine monophosphate (cGMP), a secondary messenger affecting cell growth and division, in activated leukocytes. High oxygen concentration results in reduced expression of endothelial adhesion molecules and increases NO production, which also inhibits neutrophil adhesion. HBOT causes the production of reactive nitrogen species (RNS), which impairs β2 integrin function. Impaired β2 integrin function decreases neutrophil adhesion to the vascular endothelium, while maintaining immune function (Atochin *et al*. 2000, Kihara *et al*. 2005).

HBOT enhances the function of leukocytes

White blood cells use oxygen-independent and oxygen-dependent mechanisms to kill microorganisms. HBOT's effects on neutrophil activity result in decreased inflammation within damaged tissue and increased bactericidal capabilities. In tissues where oxygen tensions fall below 30 to 40 mmHg, leukocytes lose their effectiveness against Gram-positive and Gram-negative organisms, allowing bacteria to flourish (Goldman 2009, Lam *et al*. 2017). HBOT improves phagocytosis impaired by hypoxia. HBOT enhances granulocyte production of antimicrobial agents such as free radicals and reactive/toxic oxygen species. Phagocytosis of pathogens results in an "oxidative burst" comprised of oxygen radicals such as hydroxyl radicals, peroxides, and superoxides to kill microorganisms. The oxidative burst increases the oxygen consumption of the leukocyte 30 times; therefore, production of these oxygen radicals requires an oxygen-rich environment, which is restored with HBOT. Anaerobic bacteria are very susceptible because they have poor defences against free radicals and other toxic oxygen species created by neutrophils in that environment (Cimşit *et al*. 2009, Thom 2009).

Macrophages, like neutrophils, are affected by tissue oxygen tension. In hypoxic conditions, macrophages are unable to scavenge effectively or produce peroxides. Hypoxia induces macrophages to produce the inflammatory cytokines TNF-alpha, IL-1, IL-8 and intracellular adhesion molecule-1, which decrease response to infection (Tazzyman *et al*. 2014). HBOT increases oxygen to normal levels so that macrophages can function normally.

Direct antibacterial and antifungal effects of HBOT

Tissue contamination often compromises healing. This can be due to infection with single or multiple pathogens, fungi or opportunistic introduced bacteria. Hyper-oxygenated tissue produces a toxic environment for bacterial and fungal growth (Kahle & Cooper 2017, Sanford *et al*. 2018). Increased superoxide levels, both intra- and extracellularly, lead to increased production of hydrogen peroxide and other toxic oxygen radicals, all of which impair microbial and fungal metabolic reactions (Semenza 2001, Schroedl *et al*. 2002). HBOT has been used as an adjunct therapeutic in infection control in both fungal and bacterial infections, in species such as *Aspergillus fumigatus*, *Rhinocerebral mucormycosis*, *Candida albicans*, *Clostridium difficile*, and *Clostridium perfringens*, among others (Demello *et al*. 1973, Gudewicz & Davis 1987, Couch & Mader 1988, Bitterman 2007, Kaide & Khandelwal 2008, Tragiannidis 2009, Dhingra & Cramer 2018). *C. perfringens*, in particular, is an anaerobe easily killed in the hyperbaric environment (Mathieu & Wattel 2006).

HBOT has direct bacteriostatic and bactericidal effects against Gram-positive, Gram-negative aerobic and anaerobic microorganisms, equal to some antibiotics. Anaerobic microorganisms cannot survive the amount of reactive oxygen species (ROS)/oxygen-based free radicals created in hyperoxic environments because they lack the superoxide dismutase (the superoxide degrading enzyme) and catalase (the hydrogen-peroxide degrading enzyme) (Fridovich 1973). Free radicals oxidise proteins and membrane lipids, damage DNA and inhibit metabolic functions (protein and nucleic acid synthesis) needed for bacterial growth and so are lethal for microorganisms that lack protection from them (particularly anaerobes).

When HBOT is utilised in the early course of infection with appropriate antibiotics and surgical attention, many of the deleterious effects of bacterial endotoxin release are minimised (Vishwanath 2012). HBOT, combined with proper surgical debridement and antibiotics, greatly enhances healing and speeds clearing of infection.

Indirect antibacterial and antifungal effects of HBOT

Several classes of antibiotics require oxygen for transport over cellular membranes. Therefore, antibiotic potency can be enhanced in a hyper-oxygenated environment. Specifically, HBOT aids in the penetration and efficacy of aminoglycosides, fluoroquinolones, Amphotericin B and the antimetabolites/sulfonamides trimethoprim, sulfamethoxazole and sulfasoxazole (Vishwanath 2012). Though this synergy can be utilised to encourage

recovery, HBOT can also be used alone, in the instance of antibiotic-resistant bacteria (Goerger *et al.* 2016).

Increased distance of oxygen diffusion

HBOT causes vasoconstriction while simultaneously increasing the oxygen available to deprived injured tissues. In vascular circulation, oxygen travels through capillary walls and diffuses into the surrounding tissue beyond capillary walls. The delivery of oxygen to damaged cells is essential for healing (Strauss 2002, Buettner 2007). During HBOT at 3 ATA, it has been estimated that oxygen can diffuse up to four times the distance from capillaries, as compared to ambient conditions, affording increased oxygenation to damaged tissue, and enhanced cellular repair (Krogh 1919). Paired with the neovascularisation effects of hyperbaric oxygen, the increased oxygen diffusion radius has significant implications for wound healing. The combined benefits of increased oxygen partial pressure, oxygen tissue diffusion, reduction of edema, and decreased inflammation outweigh concerns for reduced blood flow due to arteriolar vasoconstriction.

Reduction of ROS in ischemia/reperfusion injury

Ischemia/reperfusion injury occurs in many disease states when the restoration of blood flow to tissues causes dangerous vascular and tissue damage, further impairing blood flow and healing. Ischemia reperfusion (IR) injuries are a common source of debilitation and potential death – usually as a result of major trauma and the associated shock, resuscitation or stroke (Francis & Baynosa 2017). IR injury can occur in a variety of tissues, from inciting events such as cranial trauma, stroke, carbon monoxide toxicity, muscular trauma and compartment syndrome, aortic thromboembolism, intestinal obstruction, gastric dilatation–volvulus and pancreatitis.

Ischemia is a prolonged period of interrupted blood flow to tissue, which results in poor oxygenation. Due to the lack of oxygen supply, cells transition to anaerobic metabolism, which yields increased lactate levels, decreased ATP levels and reduced ATP-dependent ion transport in the cell. As ions build up, cells swell and eventually burst. During reperfusion, the restoration of blood flow actually leads to progressive vascular damage and an expanding area of poor blood flow. This resultant tissue damage is referred to as IR injury.

The return in blood flow causes neutrophil adhesion to the venules, oxygen radical production and venule damage. It also causes the release of vasoactive agents that produce arteriolar vasoconstriction leading to a second episode of ischemia and tissue hypoxia. The increase in the number of ROS causes damage to proteins and DNA and contributes to the inflammatory response (Francis & Baynosa 2017). This inflammatory response causes the release of cytokines and may lead to eventual cell death. It also restarts the activity of ATP-dependent ion pumps, which go into overdrive, causing calcium ion overload, apoptosis and cellular death (Yellon 2007). This response can cause cellular death for days after the reperfusion, which results in extensive, lasting tissue damage (Zhao *et al.* 2000).

HBOT is effective in reducing the effects of IR injuries by tempering the initial tissue hypoxia, decreasing neutrophil adhesion to the venules, reducing ROS activity and producing an anti-inflammatory effect (Buettner 2007, UHMS 2014). HBOT protects tissues from the harmful effects of toxic oxygen radicals or ROS by providing enough oxygen for the re-perfused tissues to generate NO to bind the ROS. HBOT also antagonises lipid peroxidation of cell membranes from toxic oxygen radicals by preventing the conversion of endothelial xanthine dehydrogenase to xanthine oxidase, an essential step required for lipid peroxidation.

HBOT also interferes with the neutrophil adhesion associated with IR injury by inhibiting beta-2-integrin (CD18) expression via a reduction in cGMP levels (Francis & Baynosa 2017). The increase prevalence of NO also interferes with neutrophil endothelial adhesion, also via inhibition of CD18. The benefit of HBOT in IR injury is largely mediated by the presence of NO and the subsequent effects on beta-2-integrin.

Neovascularisation

Destruction or injury of tissues results in damage of the supporting blood vessels. Neovascularisation is important to wound healing, as the vasculature is the conduit for incoming nutrients and the removal of waste products, debris and foreign microorganisms. HBOT helps create collateral blood flow by increased angiogenesis (new vessel growth budding from existing intact vessels) and vasculogenesis (when bone marrow-derived endothelial precursor cells populate the tissue and differentiate and grow into new vessels) (Niinikoski 2004).

Elevated cellular oxygen (O_2) leads to an increase in ROS and RNS in the tissue (Thom 2009). These contribute to growth factor synthesis, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF), which promote angiogenesis and neovascularisation.

One of these RNS is NO, which is known to play a particular role in enhancing VEGF-induced endothelial cell growth and angiogenesis by regulating extracellular kinases involved in the process (Parenti *et al.* 1998, Fukumura *et al.* 2001).

Hypoxia-inducible factors (HIFs) are stimulated under hypoxic conditions during oxidative stress. Free radicals are necessary for HIF expression (Semenza 2001, Schroedl *et al.* 2002). Therefore, the reactive species generated during HBOT enhance the synthesis and downstream effects of HIF, which includes gene regulation to enhance neovascularisation.

Stem cell induction/promotion properties

Though there are pharmaceutical options for increasing stem cell levels in the body such as erythropoiesis-stimulating agents (erythropoietin, darbepoetin) and colony-stimulating factors (CGFs) such as granulocyte CSF (G-CSF), one of the most exciting effects of HBOT is the increased mobilisation of stem cells in circulation of patients. This finding could explain much of what has been seen in the physiologic activity of HBOT.

A single 2-hour exposure to HBOT at 2 ATA doubles circulating CD34+ progenitor stem cells (primordial cells targeted to salvage and restore damaged structures); and at approximately 40 hours of HBOT circulating CD34+ cells increase eightfold

(800%) (Thom *et al.* 2006). This groundbreaking study offers a new, safe, therapeutic option for stem cell proliferation with little risk of side effects, as the pharmaceutical options might have. This was demonstrated to be particularly useful in diabetic patients where increases in stem cells were directly correlated to improved refractory lower extremity neuropathic ulcers. NO synthase activity was found to be acutely increased in patient's platelets following HBOT and remains elevated for at least 20 hours. HBOT-aided healing may be due to increased vasculogenic stem cell mobilisation to skin wounds.

In vitro studies reveal an increase in MSCs in response to HBOT (Shyu *et al.* 2008, Dhar *et al.* 2012). Stem progenitor cells (SPCs) are necessary for the stimulation of angiogenesis. Certain conditions, including diabetes and radiation in human medicine, reduce SPC mobilisation (Thom 2012). However, HBOT has also been shown to increase SPC mobilisation via the production of NO. Stimulation of bone marrow-derived mesenchymal stem cells (MSCs) is dependent on ROS concentrations. SPCs also play a role in the activity of HIFs, which then recruit growth factors involved in neovascularisation and vasculogenesis (Milovanova *et al.* 2008, Milovanova *et al.* 2009).

Pain medication

Although pain medication is not technically a part of wound healing, time to function and alleviation of pain in the process is very important. In veterinary medicine, quality of life is deemed more important than the length of time a pet lives; therefore, reducing pain is considered extremely important when managing any patient. Hyperbaric oxygen can be a component of a multimodal pain control plan. There is increasing evidence for HBOT as an effective adjunctive treatment for management of neuropathic pain (Zhao *et al.* 2014, Han *et al.* 2017). Early studies in rodents indicate that HBOT could inhibit the mTOR pathway and induce autophagic flux, which is known to reduce neuropathic pain (Marinelli *et al.* 2014, Yong-Da *et al.* 2017). Pain associated with the spinal nerve ligation (SPL) model and with chronic constriction injury in rats models has been mediated by HBOT. It has also been shown as efficacious in managing pain in an arthritis model, decreasing joint pain (Wilson *et al.* 2007). A study done in mice suggests that HBOT causes a NO mediated effect on opioid receptors resulting in a lasting antinociceptive response (Chung *et al.* 2010).

In addition to the above possible mechanisms for reduction of pain, using hyperbaric oxygen will decrease the previously discussed inflammation-associated swelling, edema and infection, which also influence pain.

Longer-term effects (days to weeks)

Effects of HBOT are prolonged after HBOT sessions end and tissue oxygen levels return to pretreatment values. Gas and tissue levels of O_2 rapidly return to baseline after HBOT. However, some effects of the treatment, especially after many successive treatments, are lasting. A number of days following injury, there is migration of fibroblasts (cells responsible for collagen production) into the areas of damage (Niinikoski 2004). These cells then divide and replicate producing large amounts of collagen a connective tissue that acts as the building block for the healing of tissue and wounds. Then, new capillaries can grow into this matrix. Likewise, the effects of osteogenesis, angiogenesis and neovascularisation are lasting effects in the healing tissue and bone.

DELIVERING HBOT TO VETERINARY PATIENTS

HBOT is emerging as an effective and valuable tool in the care of veterinary patients, especially as an adjunctive therapy to presently standard-of-care protocols for conditions such as ischemia–reperfusion injury and complicated wounds (Braswell & Crowe 2012). Delivering HBOT to veterinary patients requires intimate familiarity with the hyperbaric oxygen equipment and safety guidelines (Lyman 2015). Although this is not a technically difficult procedure, there are many safety principles and guidelines that must be followed. This is not within the scope of this manuscript; however, its importance cannot be emphasised enough.

In most veterinary clinical situations, HBOT utilises 100% oxygen. The duration of treatment and the level of pressure (ATA) chosen are variables at the discretion of the prescriber who is planning treatment. The decisions on duration and pressure may be based upon multiple factors, including prior published studies, facility standards of care, the patient's unique clinical situation and the clinician's experience. Clinical considerations include medical history, current patient status, medications (e.g. potential enhancement of narcotic effects, corticosteroids, chemotherapeutics) or confounding restrictions (presence of pulmonary bulla, significant hypothermia, severe middle ear disease, concern regarding seizures, ongoing need for critical monitoring and treatments). It is common for initial treatment plans to be revised in response to changing circumstances and patient factors. Treatment plans would normally include a selected ATA, time to reach pressure, duration time at pressure, time to return to normobaric pressure and an initial schedule of anywhere from 1 to 30 treatments depending upon the diagnoses and circumstances (Shmalberg *et al.* 2015). Treatment plans are commonly once or twice daily for 50 to 90 minutes. Common treatment pressures are usually within a range of 1.3 to 2.8 ATA (Shmalberg *et al.* 2015). Treatment number and frequency will vary tremendously based on the cases at hand, owners' financial and time constraints and the schedule of the facility.

Class C chambers, as designated for veterinary use, are the equivalent of Class B human monoplace chambers. Fig. 3 demonstrates the placement of two veterinary patients in a Class C chamber. This affords the animal patient a comfortable contained environment for delivery of hyperbaric oxygen. Animal HBOT chambers have a variety of features that address monitoring and safety of the patient. Features such as view ports or transparent acrylic walls allow for continual patient monitoring and visualisation. An intercom system allows for verbal communication that might be calming for a patient during treatment. Most chambers come with digital displays, which give continuous information on chamber gas content (percent of O_2 and CO_2), humidity and

FIG 3. Placement of two veterinary patients within a Class C chamber

temperature inside the chamber. They also allow for the adjustment of CO_2 level via adjustments of the oxygen flow rate. For larger patients who exhale higher volumes of CO_2, this becomes important. Passive addition of moisture in the chambers (e.g. water trays, wet towels) can be used to maintain humidity.

Some chambers are large enough to house large dogs, including Great Danes and Newfoundlands. Most chambers can house multiple smaller animals (via separation acrylic chambers) when the same treatment regimen is to be employed in multiple patients. If needed, intravenous medications and fluids can be given during treatments in some chambers.

Thousands of HBOT treatments are conducted each day around the world in both human and veterinary medical practice. Very few accidents have occurred in the history of hyperbaric medicine, and these are preventable with proper machine housing and maintenance. In the USA, several organisations have provided safety guidelines for chamber design, maintenance, education, training and facilities. In the 2019 version of the National Fire Protection Association's publication of NFPA 150 – Fire and Life Safety in Animal Housing Facilities Code, a section was added that addresses hyperbaric chambers (NFPA 2019).

Given the risk of pure oxygen gas line hyperbaric chambers are grounded to dissipate static, humidity levels are monitored during treatments, and strict protocols for the materials permitted within the chamber must be enforced via protocols and operator training. Pre-treatment procedures include thorough equipment and patient inspection. Adherence to proper preparation and treatment methods is the goal to ensure patient and operational safety.

CLINICAL USES IN VETERINARY PRACTICE

In veterinary medicine, scientific evaluation of HBOT is scarce; therefore, applications and protocols are largely adopted from human studies and anecdotal reports from user experiences. HBOT has been successfully used in veterinary practice as adjunctive treatment of traumatic, acute and/or emergency situations, including necrotizing fasciitis, thermal burns, crush injuries, carbon monoxide poisoning, enhanced healing of complicated wounds, gas gangrene, intracranial abscess, compromised skin flaps and grafts and radiation injury (Edwards 2010a, Edwards 2010b, Geiser 2016, Birnie *et al.* 2018, Latimer *et al.* 2018). HBOT has been used to treat humans and animals for acute brain and spinal injuries, situations of neuropraxic trauma and post-concussive brain trauma, aortic thromboembolisms and fibrocartilaginous embolisms (FCEs).

Ischemia reperfusion injury

As discussed above, the return in blood flow and subsequent re-oxygenation to ischemic tissue can cause tissue damage. Treatment of IR Injury is a common indication in human medicine, and these types of injuries encompass a number of the UHMS-supported indications for HBOT (Weaver 2014). At this time, there are several published studies to likewise support the treatment of IR injuries in a veterinary practice with HBOT.

A study was performed in 80 rabbits with spinal cord IR injury. Following HBOT, the animals were assessed for neurological function and histopathology. The treatment group had statistically significantly improved neurological function and histopathological scores, as compared to control (Ilhan *et al.* 2013).

IR injury can lead to liver damage, due to the excess neutrophils in circulation. In another study of hind limb IR injury, rabbits were given 90-minute hyperbaric oxygen treatments (Lukiswanto *et al.* 2017). In this study, histopathological improvements were observed, including fewer necrotic lesions, and less inflammation at the site of injury (Lukiswanto *et al.* 2017). Further, the HBOT was protective of hepatocyte function, as evidenced by reduced necrotic lesions, lower ALT enzymatic values and portal inflammation in the treatment groups (Lukiswanto *et al.* 2017).

Another study was conducted in 32 rats after 60 minutes of acute mesenteric ischemia, followed by reperfusion (Açiksari *et al.* 2019). The study was intended to assess the potential benefit of HBOT on the healing process of the rats' intestinal mucosa. The HBOT treatment results showed that both pre- and

post-ischemia-induced lesion size was lessened and that there was increased cell viability via reduction in caspase-3, increased CD34 stem cells and increased VEGF.

Venomous/infected bite wounds

A particularly relevant application of HBOT in veterinary practice is in the healing of complicated or necrotizing bite wounds. A number of studies demonstrate improved vascularisation and epithelialisation, increased tissue oxygen and a reduction of bacteria in venomous bite wounds (e.g. both spider and snake bites) following a course of HBOT.

A randomised study was conducted in rabbits, which had been injected with brown recluse venom. The rabbits were treated daily or twice daily for 7 days at 2 ATA for 60-minute sessions. Treatments were initiated 3 days after envenomation to simulate the lag time between bite and initiation of clinical intervention. The pretreatment wounds were observed to be swollen, red, and ischemic. In some cases, the wounds exhibited rashes and/or cutaneous ulcer formation. In the treatment group, which received twice daily HBOT, a histological evaluation of tissue excised from the wounds revealed re-epithelialisation (Strain *et al.* 1991). Conversely, necrotic cavities and excess inflammation were observed in both the control group and the group with only one treatment per day. In this particular clinical scenario, there were benefits to wound healing from a twice daily treatment regimen following venomous bites. Additionally, histological evaluations performed on day 24 indicated that the effects of the HBOT between groups were still discernible more than 2 weeks after the final treatment.

Complicated and necrotizing wounds

Chronic, persistent wounds can exhibit an excess inflammatory response, which inhibits healing. HBOT stimulates a downregulation of this excess inflammatory response, in order to create a more conducive environment for the development of healthy tissue (Thom 2009). HBOT has been shown to downregulate pro-inflammatory cytokines, mediators for the body's immune response, and to upregulate several growth factors such as vascular endothelial cell growth factor (VEGF), a signalling molecule that stimulates angiogenesis, the development of new blood vessels (Butler 2006, Thom 2009). Healthy granulation tissue is promoted via increased NO levels in wound fluid following HBOT (Boykin *et al.* 2007). This effect is added to other positive effects of HBOT in complicated wounds via positive effects of angiogenesis, moderated inflammatory responses, decreased edema and antimicrobial responses.

For example, necrotizing soft-tissue infections, gangrene and need for enhancement of healing in problem wounds, such as diabetic foot ulcers, are all common indications for HBOT in humans (Weaver 2014). The mechanism behind these indications entails the reduction of edema, leukocyte adhesion, hypoxia and methane accumulation in the necrotizing or infected tissue. As discussed above, the antimicrobial properties of HBOT are an additional benefit to the therapy. HBOT can also enhance antibiotic distribution within infected tissue, as some antibiotics require oxygen for transport across cellular membranes.

Several studies in mammals have demonstrated improved vascularisation and blood flow in complicated wounds after successive HBOT. In a study of rats, laser Doppler flowmetry (LDF) was used to measure blood flow in healing soft tissue. Not only did perfusion increase during the treatments, elevated perfusion was observed weeks after the cessation of therapy, indicating that the treatments had lasting effects in tissue oxygenation (Klemetti *et al.* 2005).

HBOT is recommended as an adjunctive tool concurrent with debridement, antibiotics and other standard-of-care components of complicated and necrotizing wound management.

Graft survival

HBOT has shown particular efficacy in wound healing in instances where skin and bone grafting or tissue transplantation were required (Al-Waili *et al.* 2006). HBOT initially enhances graft survival by decreasing the hypoxia and effects of ischemia as described above. Then HBOT has been shown to bolster fibroblast function, and augment collagen synthesis (Baynosa & Zamboni 2012).

HBOT has been studied in wound grafting in both dogs and cats (Smith *et al.* 1995, Kerwin *et al.* 2000). In a study of bone grafting for ulnar defects in 12 mature cats, six cats were treated at 2 ATA for 90 minutes once per day for 14 days. The control group did not receive HBOT. HBOT was shown to increase median percentage of bone formation in the treatment group, as compared to control (Kerwin *et al.* 2000). The bones were marked with fluorescent labels. At 5 weeks, the treatment group showed more distance between fluorescent labels than did the control group, indicating increased bone formation.

Thermal burns

Burns, either from sun exposure, scalding liquids, steam, flames, hot gasses or hot metal, can result in complicated, necrotic wounds, resistant to healing. HBOT has been successfully employed in wound healing for thermal burns in a number of species (Weaver 2014). A combination of antibiotics, debridement and HBOT is supported in literature (Frank *et al.* 2015). HBOT use in burn wound management is founded in its ability to attenuate infection, to enhance neovascularisation and collagen synthesis via the recruitment of growth factors, to stimulate ROS and RNS production, to reduce edema and to enhance oxygen delivery to ischemic tissue (Dinar *et al.* 2008, Choudhury 2018).

Bone injuries

HBOT is a well-established therapies for crush injuries, bony necrosis, osteomyelitis and open fractures in human medicine (Weaver 2014). Likewise, there have been a number of studies conducted to assess the utility of HBOT in osteogenesis, bone healing and bone graft survival for veterinary use cases. HBOT increases osteoclast and osteoblast production, proliferation, differentiation and activity. Bone healing, as well as resorption of necrotic bone by osteoclasts, is enhanced with HBOT. A recent study in mice suggests that the proliferative effects of HBOT on osteoblasts may contribute to the recruitment of osteo-

blasts at fracture sites and during digit regeneration (Sammarco *et al.* 2015).

In a randomised study of 48 rabbits with open tibial fractures, HBOT was shown to reduce the inflammatory response and to promote osteocyte proliferation and subsequently, to promote fracture healing with a statistically significant difference (Chen *et al.* 2017). The rabbits were treated daily for 2 weeks at 2 ATA for 50 minutes. The HBOT group exhibited a reduced inflammatory reaction and reperfusion injury and promoted osteocytic proliferation and fracture healing (Chen *et al.* 2017).

Another study of HBOT influence on bone healing involved a bone segment transplantation surgery model for cleft palate repair in six beagle dogs. This randomised small study assessing distraction osteogenesis had three patients treated with HBOT for 20 days and the other three dogs randomised to a control group (Kudoh 2008). The researchers monitored blood flow throughout the course of therapy, and the dogs were sacrificed on day 100. Blood flow recovered 30 days sooner in the HBOT group, and bone mineral density was statistically significantly higher in the HBOT group, as compared to control. Additionally, more bone had developed in the treatment group than in the control group. This may then have positive implications for the value of HBOT as adjunctive support for healing of various crush injuries and other types of fractures (Kudoh 2008).

Mechanical trauma and haemorrhagic shock

Large studies have been conducted in dogs on the effects of HBOT in severe mechanical trauma and haemorrhagic shock. In one study of 80 dogs, 4 to 5, fifty-minute HBOT sessions were shown to enhance the chances of survival (Magomedov 1990). In another study of 56 dogs with haemorrhagic and traumatic shock, the patients were treated at 2 ATA for 1 hour. It was shown that early intervention with HBOT improves chances of survival, while delayed time to treatment did not show significant improvements in survival (Sherman *et al.* 1989). This study demonstrates the value of timely HBOT in patients with haemorrhagic shock.

REAL-WORLD EXPERIENCE

In our veterinary practice in Commack, New York, HBOT is utilised for a wide diversity of the conditions mentioned above. Fig. 4 contains a series of pre-treatment and post-treatment images from practice, depicting the treatment of (A/B) osteomyelitis and cellulitis; (C/D) closed airway following a sting and (E/F) edema following plasma transfusion.

CONCLUSION

HBOT shows promise in the management of a number of animal species for treatment of traumatic or complicated wounds, ischemia–reperfusion injury, burns, venomous or infected bites, concussive and crush injuries, compromised surgical graft and flap healing, among a multitude of other similar conditions. The rationales described have included improved wound healing, management of infection via toxic oxygen rich environment for anaerobic and microaerophilic organisms, inhibition of some bacterial mechanisms of resistance to antibiotics, augmenting

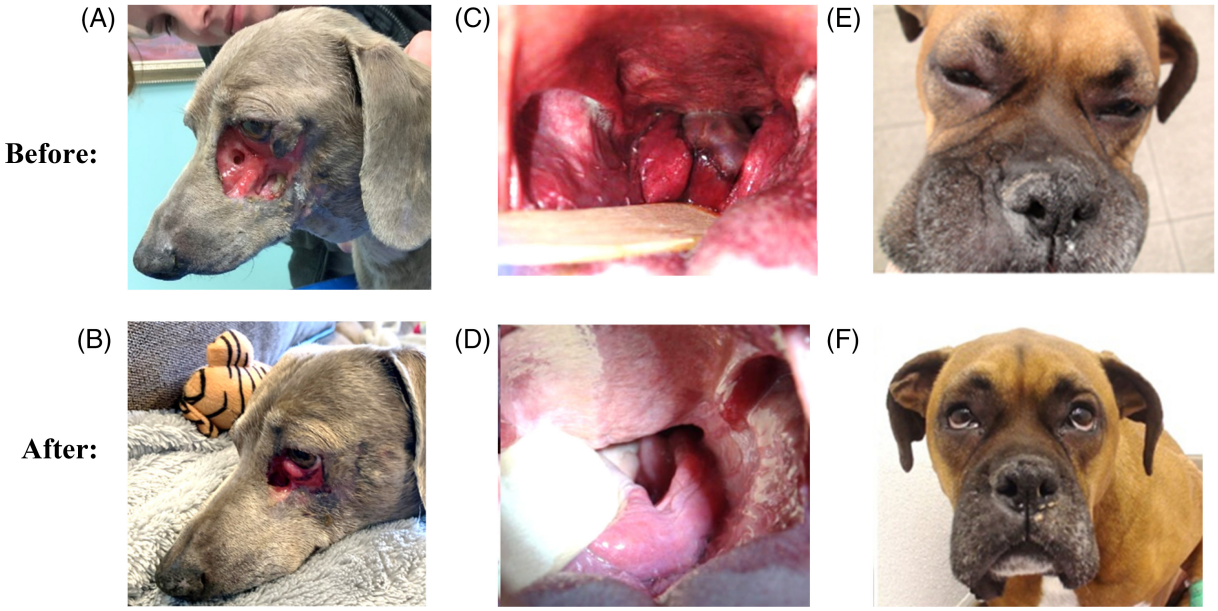

FIG 4. (A) 5-year-old female dachshund presented with 2-year-old non-healing osteomyelitis and cellulitis. Hyperbaric oxygen therapy (HBOT) was prescribed daily for 5 days and every other day for an additional 10 days. Each hyperbaric oxygen treatment was 15 minutes to reach 2 ATA pressure, 1 hour at 2 ATA and 15 minutes to return to normal atmospheric pressure (1 ATA). (B) After a surgical repair and 15 one-hour HBOT sessions, wound edges moved inward, tissue healing was observed and patient has no inflammation and no pain. (C) 2-year-old male Labrador retriever presented with completely closed airway from a sting reaction. (D) Following a tracheostomy and a single 1-hour HBOT treatment at 2 ATA, the patient's airway became patent, and inflammation and swelling were reduced. (E) 6-year-old boxer presented with post-op edema following plasma transfusion for necrotizing pancreatitis. (F) After 1 hour of HBOT as the only medical intervention, immediate reduction in facial swelling was observed

angiogenesis and neovascularisation, fibrogenesis and reducing inflammation.

HBOT is intended to be an adjunctive treatment concurrently applied with traditional medical and surgical approaches. Hyperbaric oxygen is unlikely to significantly help in natural healing of healthy, uncompromised tissues (Mutluoglu *et al.* 2016). Studies measuring the healing speed of healthy tissue would not likely show improvement using HBOT. Rather, HBOT is most indicated in complicated situations of wound healing, such as when oxygen levels in the tissue are compromised by infection, ischemia–reperfusion injury, severe swelling, necrosis or other sources of oxygen depletion.

Applying HBOT in an earlier and more consistent manner could improve short- and long-term outcomes of the above situations by reducing the need for more invasive or additional procedures, reducing length of stay (LOS) in the hospital, reducing long-term care and possibly reducing drug requirements.

The authors also hope this information may stimulate interest in the design of future, prospective studies for the various clinical situations described. Such studies need to be designed properly, with adequate power to generate statistical significance and meaningful recommendations to other providers.

Conflict of interest

The authors are unpaid consultants for Sechrist and participate on their medical advisory board. Sechrist provided formatting assistance for this manuscript. No conflicts of interest to be disclosed.

References

Açiksari, K., Eğin, S., Hepgül, G., *et al*. (2019) Protective effect of hyperbaric oxygen treatment on rat intestinal mucosa after mesenteric ischaemia and reperfusion. *Diving and Hyperbaric Medicine* **49**, 253-258

Al-Waili, N., Butler, G. J., Petrillo, R. L., *et al*. (2006) Hyperbaric oxygen and lymphoid system function: a review supporting possible intervention in tissue transplantation. *Technology and Health Care* **14**, 489-498

Atochin, F. D., Demchenko, I. T. & Thom, S. R. (2000) Neutrophil sequestration and the effect of hyperbaric oxygen in a rat model of temporary middle cerebral artery occlusion. *Undersea and Hyperbaric Medicine* **27**, 185-190

Baynosa, R. & Zamboni, W. A. (2012) The effect of hyperbaric oxygen on compromised grafts and flaps. *Undersea and Hyperbaric Medicine* **39**, 857-865

Benkő, R., Miklós, Z., Ágoston, V. A., *et al*. (2019) Hyperbaric oxygen therapy dampens inflammatory cytokine production and does not worsen the cardiac function and oxidative state of diabetic rats. *Antioxidants* **8**, 607

Benson, M. L., Osborne, B. A. & Granowitz, E. V. (2003) Hyperbaric oxygen inhibits stimulus-induced proinflammatory cytokine synthesis by human blood-derived monocyte-macrophages. *Clinical and Experimental Immunology* **134**, 57-62

Birnie, G. L., Fry, D. R. & Best, M. P. (2018) Safety and tolerability of hyperbaric oxygen therapy in cats and dogs. *Journal of the American Animal Hospital Association* **54**, 188-194

Bitterman, H. (2007) Hyperbaric oxygen for invasive fungal infections. *Israel Medical Association Journal* **9**, 387

Boerema, I. (1959) Life without blood. *Journal of Cardiovascular Surgery* **13**, 133-146

Boykin, J., Joseph, V. & Baylis, C. (2007) Hyperbaric oxygen therapy mediates increased nitric oxide production associated with wound healing: a preliminary study. *Advances in Skin and Wound Care* **20**, 382-388

Braswell, C. & Crowe, D. T. (2012) Hyperbaric oxygen therapy. *Compendium: Continuing Education for Veterinarians* **34**, E1-E5

Buettner, D. W. (2007) Hyperbaric oxygen therapy in the treatment of open fractures and crush injuries. *Emergency Medicine Clinics of North America* **25**, 177-188

Butler, N. S. A.-W. A. G. J. (2006) Effects of hyperbaric oxygen on inflammatory response to wound and trauma: possible mechanism of action. *ScientificWorldJournal* **6**, 425-441

Chen, X., Cheng, X., Ma, W., *et al*. (2017) Effects of hyperbaric oxygen therapy on open tibial fractures in rabbits after transient seawater immersion. *Undersea and Hyperbaric Medicine: Journal of the Undersea and Hyperbaric Medical Society, Inc* **44**, 235-242

Choudhury, R. (2018) Hypoxia and hyperbaric oxygen therapy: a review. *International Journal of General Medicine* **11**, 431-442

Chung, E., Zelinski, L. M., Ohgami, Y., *et al*. (2010) Hyperbaric oxygen treatment induces a two-phase antinociceptive response of unusually long duration in mice. *The Journal of Pain* **11**, 847-853

Cimşit, M., Uzun, G. & Yildiz, S. (2009) Hyperbaric oxygen therapy as an anti-infective agent. *Expert Review of Anti-Infective Therapy* **7**, 1015-1026

Couch, F. T. & Mader, J. T. (1988) Rhinocerebral mucormycosis with cerebral extension successfully treated with adjunctive hyperbaric oxygen therapy. *Archives of Otolaryngology – Head and Neck Surgery* **114**, 791-794

Demello, F. J., Haglin, J. J. & Hitchcock, C. R. (1973) Comparative study of experimental *Clostridium perfringens* infection in dogs treated with antibiotics, surgery, and hyperbaric oxygen. *Surgery* **73**, 936-941

Dhar, M., Neilsen, N., Beatty, K., *et al*. (2012) Equine peripheral blood-derived mesenchymal stem cells: isolation, identification, trilineage differentiation and effect of hyperbaric oxygen treatment. *Equine Veterinary Journal* **44**, 600-605

Dhingra, J. C. B. & Cramer, R. A. (2018) Hyperbaric oxygen reduces Aspergillus fumigatus proliferation in vitro and influences in vivo disease outcomes. *Antimicrobial Agents and Chemotherapy* **62**, e01953

Dinar, S., Agir, H., Sen, C., *et al*. (2008) Effects of hyperbaric oxygen therapy on fibrovascular ingrowth in porous polyethylene blocks implanted under burn scar tissue: an experimental study. *Burns* **34**, 467-473

Dünnwald, T., Held, J., Balan, P, *et al*. (2018) Combined hyperbaric oxygen partial pressure at 1.4 bar with infrared radiation: a useful tool to improve tissue hypoxemia? *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research* **24**, 4009

Edwards, M. L. (2010a) Hyperbaric oxygen therapy. Part 1: history and principles. *Journal of Veterinary Emergency and Critical Care* **20**, 284-288

Edwards, M. L. (2010b) Hyperbaric oxygen therapy. Part 2: application in disease. *Journal of Veterinary Emergency and Critical Care* **20**, 289-297

Eming, S. A., Krieg, T., Davidson, M., *et al*. (2007) Inflammation in wound repair: molecular and cellular mechanisms. *Journal of Investigative Dermatology* **127**, 514-525

Francis, A. & Baynosa, R. (2017) Ischaemia-reperfusion injury and hyperbaric oxygen pathways: a review of cellular mechanisms. *Diving and Hyperbaric Medicine* **47**, 110-117

Frank, L., McCormick, K. A., Donnell, R. L., *et al*. (2015) Dorsal black skin necrosis in a Vietnamese pot-bellied pig. *Veterinary Dermatology* **26**, 64-67

Fridovich, E. M. G. A. I. (1973) Induction of superoxide dismutase by molecular oxygen. *Journal of Bacteriology* **1114**, 543-548

Fukumura, G. T., Kadambi, A., Izumi, Y., *et al*. (2001) Predominant role of endothelial nitric oxide synthase in vascular endothelial growth factor-induced angiogenesis and vascular permeability. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 2604-2609

Gasier, H. G., Demchenko, I. T., Zhilyaev, S. Y., *et al*. (2018) Adrenoceptor blockade modifies regional cerebral blood flow responses to hyperbaric hyperoxia: protection against CNS oxygen toxicity. *Journal of Applied Physiology* **125**, 1296-1304

Geiser, D. R. (2016) Hyperbaric oxygen therapy in equine rehabilitation: putting the pressure on disease. *The Veterinary Clinics of North America. Equine Practice* **32**, 149-157

Goerger, E. H., Savini, H., Coulange, M., *et al*. (2016) Anti-infective therapy without antimicrobials: apparent successful treatment of multidrug resistant osteomyelitis with hyperbaric oxygen therapy. *ID Cases* **6**, 60-64

Goldman, R. J. (2009) Hyperbaric oxygen therapy for wound healing and limb salvage: a systematic review. *Physical Medicine and Rehabilitation* **1**, 471-489

Goldstein, L. J., Gallagher, K. A., Bauer, S. M., *et al*. (2006) Endothelial progenitor cell release into circulation is triggered by hyperoxia-induced increases in bone marrow nitric oxide. *Stem Cells* **24**, 2309-2318

Granger, S. E. (2010) Chapter 7. Leukocyte–endothelial cell adhesion. In: *Inflammation and the Microcirculation*. San Rafael, CA: Morgan & Claypool Life Sciences

Gudewicz, J. T. M. & Davis, C. P. (1987) Combined effects of hyperbaric oxygen and antifungal agents on the growth of *Candida albicans*. *Aviation, Space, and Environmental Medicine* **58**, 673-678

Han, G., Liu, K., Li, L., *et al*. (2017) Effects of hyperbaric oxygen therapy on neuropathic pain via mitophagy in microglia. *Molecular Pain* **13**, 174480691771086

Hardy, K., Thom, S. R. & Neumann, T. (2008) Chapter 4: the physics of hyperbaric oxygen therapy. In: *Physiology and Medicine of Hyperbaric Oxygen Therapy*. Philadelphia, PA: Saunders Elsevier

Ilhan, A. M., Ozpak, B., Gunes, T., *et al*. (2013) The effect of combined hyperbaric oxygen and iloprost treatment on the prevention of spinal cord ischaemia-reperfusion injury: an experimental study. *European Journal of Cardio-Thoracic Surgery* **44**, e332-e340

Jain, K. (2017) Textbook of Hyperbaric Medicine. New York, NY: Springer. pp 610-613

Kahle, A. C. & Cooper, J. S. (2017). Hyperbaric Physiological and Pharmacological Effects Gases

Kaide, C. G. & Khandelwal, S. (2008) Hyperbaric oxygen: applications in infectious disease. *Emergency Medicine Clinics of North America* **26**, 571-595

Kerwin, S., Lewis, D. D., Elkins, A. D., *et al*. (2000) Effect of hyperbaric oxygen treatment on incorporation of an autogenous cancellous bone graft in a non-union diaphyseal ulnar defect in cats. *American Journal of Veterinary Research* **61**, 691-698

Kihara, U. S., Sakoda, M. & Aikou, T. (2005) Effects of hyperbaric oxygen exposure on experimental hepatic ischemia reperfusion injury: relationship between its timing and neutrophil sequestration. *Liver Transplantation* **11**, 1574-1580

Klemetti, E., Rico-Vargas, S. & Mojon, P. (2005) Short duration hyperbaric oxygen treatment effects blood flow in rats: pilot observations. *Laboratory Animals* **39**, 116-121

Kožár, M., Hamilton, H. & Koščová, J. (2018) Types of wounds and the prevalence of bacterial contamination of wounds in the clinical practice of small animals. *Folia Veterinaria* **62**, 39-47

Krogh, A. (1919) The number and distribution of capillaries in muscle with calculations of the oxygen pressure head necessary for supplying the tissue. *The Journal of Physiology* **52**, 409-415

Kudoh, A. (2008) Effects of hyperbaric oxygen treatment on healing of maxillary distraction osteogenesis in beagle dogs. *Kokubyo Gakkai Zasshi* **75**, 55-64

Lam, G., Fontaine, R., Ross, F. L., *et al*. (2017) Hyperbaric oxygen therapy: exploring the clinical evidence. *Advances in Skin and Wound Care* **30**, 181-190

Latimer, C. R., Lux, C. N., Roberts, S., *et al*. (2018) Effects of hyperbaric oxygen therapy on uncomplicated incisional and open wound healing in dogs. *Veterinary Surgery* **47**, 827-836

Lukiswanto, B. S., Yuniarti, W. M. & Yosis Motulo, Y. (2017) Effects of hyperbaric therapy on liver morphofunctional of rabbits (*Oryctolagus cuniculus*) after hind limb ischemia-reperfusion injury. *Veterinary World* **10**, 1337-1342

Lyman, R. (2015) Hyperbaric oxygen therapy in veterinary medicine – challenges in safety, training and usage: an editorial perspective. *Undersea & Hyperbaric Medicine* **42**, 7-8

Magomedov, A. (1990) The effect of hyperbaric oxygenation on the central hemodynamics and oxygen consumption in severe mechanical trauma. *Patologicheskaia Fiziologiia i Èksperimental'naia Terapiia* **2**, 26-28

Marinelli, S., Nazio, F., Tinari, A., *et al*. (2014) Schwann cell autophagy counteracts the onset and chronification of neuropathic pain. *Pain* **155**, 93-107

Mathieu, D. & Wattel, F. (2006) Physiologic effects of hyperbaric oxygen on microorganisms and host defences against infection. In: Handbook on Hyperbaric Medicine. New York, NY: Springer

Mihaljević, Z., Matić, A., Stupin, A., *et al*. (2018) Acute hyperbaric oxygenation, contrary to intermittent hyperbaric oxygenation, adversely affects vasorelaxation in healthy Sprague-Dawley rats due to increased oxidative stress. *Oxidative Medicine and Cellular Longevity* **2018**, 7406027

Milovanova, B. V., Sorokina, E. M., Moore, J. S., *et al*. (2008) Lactate stimulates vasculogenic stem cells via the thioredoxin system and engages an autocrine activation loop involving hypoxia-inducible factor 1. *Molecular and Cellular Biology* **28**, 6248-6261

Milovanova, T. N. V. M. B., Sorokina, E. M., Moore, J. S., *et al*. (2009) Hyperbaric oxygen stimulates vasculogenic stem cell growth and differentiation in vivo. *Journal of Applied Physiology* **106**, 711-728

Mutluoglu, M., Uzun, G., Bennett, M., *et al*. (2016) Poorly designed research does not help clarify the role of hyperbaric oxygen in the treatment of chronic diabetic foot ulcers. *Diving and Hyperbaric Medicine* **46**, 133-134

NFPA (2019) NFPA 150 11.3.2.2. In: *Fire and Life Safety in Animal Housing Facilities Code*. Quincy, MA: NFPA

Niinikoski, J. H. A. (2004) Clinical hyperbaric oxygen therapy, wound perfusion, and transcutaneous oximetry. *World Journal of Surgery* **28**, 307-311

Oyaizu, T., Enomoto, M., Yamamoto, N., *et al*. (2018) Hyperbaric oxygen reduces inflammation, oxygenates injured muscle, and regenerates skeletal muscle via macrophage and satellite cell activation. *Scientific Reports* **8**, 1-12

Parenti, M. L., Cui, X. L., Douglas, J. G., *et al*. (1998) Nitric oxide is an upstream signal of vascular endothelial growth factor-induced extracellular signal-regulated kinase1/2 activation in postcapillary endothelium. *The Journal of Biological Chemistry* **273**, 4220-4226

Robins, M. & Wyatt, H. A. (2020). *Hyperbaric Treatment of Ischemia Reperfusion Injury*. StatPearls. Accessed August 20, 2020

Saito, E. K. & Rhoads, C. (2003) Emergency visits to primary care veterinary hospitals. *Veterinary Focus* **25**, 18-19

Sammarco, M. C., Simkin, J., Cammack, A. J., *et al*. (2015) Hyperbaric oxygen promotes proximal bone regeneration and organized collagen composition during digit regeneration. *PLoS One* **10**, e0140156

Sanford, N., Wilkinson, J. E., Nguyen, H., *et al*. (2018) Efficacy of hyperbaric oxygen therapy in bacterial biofilm eradication. *Journal of Wound Care* **27**, S20-S28

Scallan, H. V. & Korthuis, R. (2010) Chapter 4. *Pathophysiology of edema formation*. In: Capillary Fluid Exchange: Regulation, Functions, and Pathology. Morgan & Claypool Life Sciences, San Rafael, CA

Schroedl, M. D., Budinger, G. R. & Chandel, N. S. (2002) Hypoxic but not anoxic stabilization of HIF-1alpha requires mitochondrial reactive oxygen species. *American Journal of Physiology. Lung Cellular and Molecular Physiology* **283**, L922-L931

Semenza (2001) HIF-1 and mechanisms of hypoxia sensing. *Current Opinion in Cell Biology* **13**, 167-171

Sherman, D., Sennik, V. T. & Sidenko, V. P. (1989) The efficacy of hyperbaric oxygenation in experimental and hemorrhagic shock. *Anesteziologiia i Reanimatologiia* **2**, 37-39

Shmalberg, J., Davies, W., Lopez, S., *et al*. (2015) Rectal temperature changes and oxygen toxicity in dogs treated in a monoplace chamber. *Undersea and Hyperbaric Medicine* **42**, 95-102

Shyu, K., Hung, H. F., Wang, B. W., *et al*. (2008) Hyperbaric oxygen induces placental growth factor expression in bone marrow-derived mesenchymal stem cells. *Life Sciences* **83**, 65-73

Smith, B., Hosgood, G. & Hedlund, C. S. (1995) Omental pedicle used to manage a large dorsal wound in a dog. *The Journal of Small Animal Practice* **36**, 267-270

Strain, G., Snider, T. G., Tedford, B. L., *et al*. (1991) Hyperbaric oxygen effects on brown recluse spider (*Loxosceles reclusa*) envenomation in rabbits. *Toxicon* **29**, 989-996

Strauss, B. (2002) Hyperbaric oxygen for crush injuries and compartment syndromes: surgical considerations. In: Hyperbaric Surgery Perioperative Care. North Palm Beach, FL: Best Publishing Company. pp 341-357

Tazzyman, S., Murdoch, C., Yeomans, J., *et al*. (2014) Macrophage-mediated response to hypoxia in disease. *Hypoxia* **2**, 185

Thom, S. R. (2011) Hyperbaric oxygen–its mechanisms and efficacy. *Plastic and Reconstructive Surgery* **127**, 131S

Thom, S. R. (2012) Hyperbaric oxygen – its mechanisms and efficacy. *Plastic and Reconstructive Surgery* **127** (Suppl 1), 142S-143S

Thom, S. R. (2009) Oxidative stress is fundamental to hyperbaric oxygen therapy. *Journal of Applied Physiology* **106**, 988-995

Thom, S. R., Bhopale, V. M., Velazquez, O. C., *et al*. (2006) Stem cell mobilization by hyperbaric oxygen. *American Journal of Physiology. Heart and Circulatory Physiology* **290**, H1378-H1386

Tragiannidis, A. (2009) Hyperbaric oxygen therapy and other adjunctive treatments for zygomycosis. *Clinical Microbiology and Infection* **15**, 82-86

Undersea Hyperbaric Medical Society (UHMS). (2014). 13th Edition of the Hyperbaric Oxygen Therapy Indications. Crush Injury, Compartment Syndrome And Other Acute Traumatic Ischemias

Vishwanath, S. B. A. G. (2012) Hyperbaric oxygen and wound healing. *Indian Journal of Plastic Surgery* **45**, 316-324

Weaver, L. (2014). 13th Edition of the Hyperbaric Oxygen Therapy Indications, Undersea and Hyperbaric Medical Society

Weisz, A. L., Adir, Y., Melamed, Y., *et al*. (1997) Modification of in vivo and in vitro TNF-alpha, IL-1, and IL-6 secretion by circulating monocytes during hyperbaric oxygen treatment in patients with perianal Crohn's disease. *Journal of Clinical Immunology* **17**, 154-159

Wernick, B. & Stawicki, S. P. (2020) Impaired wound healing. Treasure Island, FL: StatPearls Publishing StatPearls. Accessed August 20, 2020

Wilson, H. D., Toepfer, V. E., Senapati, A. K., *et al*. (2007) Hyperbaric oxygen treatment is comparable to acetylsalicylic acid treatment in an animal model of arthritis. *Journal of Pain* **8**, 924-930

Yellon, H. D. (2007) Myocardial reperfusion injury. *New England Journal of Medicine* **357**, 1121-1135

Yong-Da, L., Wang, Z.-B., Han, G., *et al*. (2017) Hyperbaric oxygen treatment attenuates neuropathic pain by elevating autophagy flux via inhibiting mTOR pathway. *American Journal of Translational Research* **9**, 2629-2638

Zhang, Q., Chang, Q., Cox, R. A., *et al*. (2008) Hyperbaric oxygen attenuates apoptosis and decreases inflammation in an ischemic wound model. *Journal of Investigative Dermatology* **128**, 2102-2112

Zhao, B.-S., Meng, L.-X., Ding, Y.-Y., *et al*. (2014) Hyperbaric oxygen treatment produces an antinociceptive response phase and inhibits astrocyte activation and inflammatory response in a rat model of neuropathic pain. *Journal of Molecular Neuroscience* **53**, 251-261

Zhao, N. M., Wang, N. P., Velez, D. A., *et al*. (2000) Dynamic progression of contractile and endothelial dysfunction and infarct extension in the late phase of reperfusion. *The Journal of Surgical Research* **94**, 133-144