FROM THE $HBO_2$ INDICATIONS MANUAL, 14TH EDITION:

CHAPTER 14

# Hyperbaric oxygen for thermal burns

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## ABSTRACT

A significant and consistently positive body of evidence from animal and human studies of thermal injury supports the use of hyperbaric oxygen as a means of preventing dermal ischemia, reducing edema, modulating the zone of stasis, preventing partial- to full-thickness conversion, preserving cellular metabolism and promoting healing. The vast majority of clinical reports have shown reduction in mortality, length of hospital stay, number of surgeries and cost of care. Hyperbaric oxygen has been demonstrated to be safe in the hands of those thoroughly trained in rendering this therapy in the critical care setting and with appropriate monitoring precautions. Careful patient selection is mandatory. ∎

## BACKGROUND

The National Burn Repository reviewed the combined data of acute burn admissions for the time period between 2006 through 2015. The key findings included data from 96 hospitals, 36 states, and the District of Columbia, totaling 205,033 records. Male patients outnumbered female patients considerably. The bimodal distribution with the greatest prevalence in the pediatric age range from 1-15 compromised 30% of total burns, while the adult age group from 20-59 comprised 54% of burns. Patients age 60 or older represented 14% of burn cases. More than 75% of reported total burn cases involved less than 10% total body surface area (TBSA) and resulted in a mortality of 0.6%. The mortality rates were 3.3% for all cases and 5.8% for fire/flame injuries.

Seventy-three percent of burn injuries occurred in the home. Nearly 95% of all reviewed burn injuries were identified as accidents, with 14% reported as work-related cases. Just over 2% were suspected of abuse, and 1% were self-inflicted. During the 10-year period from 2006-2015, the average length of stay for females declined from 9.3 days to 7.9 days, while that for males declined less significantly, from 9.1 to 8.8 days. The mortality rate for females declined from 4.1% to 2.9%, while in males the decline was from 3.9% to 3%.

Deaths from burn injury increased with advancing age and burn size as well as presence of inhalation injury. A 20-39% burn in patients below 60 conferred a morality rate of 2.5%. In the presence of inhalation injury, the mortality rate increased to 14%. The same injury in a 60-year-old shows a mortality of 32%, which increased to 55.8% in the presence of inhalation injury. Thus, age and inhalation injury, as well as burn size, are important factors in burn injury survival. Pneumonia was the most frequent, clinically related complication, occurring in 5.4% of fire/flame or flame-injured patients. The frequency of pneumonia and respiratory failure was greater in patients with four days or greater of mechanical ventilation. The rate of complications also increased with age.

For survivors, the length of stay was slightly greater than one day per percent total body surface area burn. For patients who died, the total hospital days were two times that of survivors on the average. However, this trend was reversed in patients with less than 20% total body surface burns. Eighty-seven percent of patients were discharged to home, while 3% were transferred to rehab facilities. Overall, charges for patients who died were three times greater than those who survived. However, this result was greatly affected by the large number of patients with less than 10% total body surface area burns. For this group, total charges averaged $257,582

KEYWORDS: hyperbaric oxygen therapy; thermal burns; treatment

for survivors, while non-survivors' charges averaged $340,474.

Burn care is extraordinarily expensive. Charges for a 50-59% total body surface area averaged $1,066,254 in 2015. A 60–70% burn averaged $1,168,006 during the same time frame. Workers compensation or automobile insurance were involved in approximately 10% of the reviewed cases, while "no information provided" or "self-insured" was indicated in 29% of cases.

Significant morbidity attaches to burn injury: pneumonia, cellulitis, respiratory failure, urinary tract infection, wound infection, and sepsis are still the most frequently reported complications adding to mortality [1]. Therapy for burns, therefore, is directed to minimizing edema, preserving marginally viable tissue in the zone of stasis, protecting the microvasculature, enhancing host defenses, and providing the essential substrates necessary to maintain viability. The ultimate goals of burn therapy include survival of the patient, rapid wound healing, minimization of scarring or abnormal pigmentation, prevention of long-term problems such as chronic pain, and cost-effectiveness. Optimal outcome, obviously, is restoration, as nearly as possible, to the pre-burn quality of life [2,4].

A recent report by Wolf et al. summarized problems and research priorities in burns for the coming decade [3]. A major and continuing clinical problem is the innate inflammatory response induced by genetic factors such as those common in neutrophils and macrophages which are massively increased while the T-cell adaptive responses are downregulated. The latter are, perhaps, responsible for late effects of severe burns such as viral and fungal infections. Hyperbaric oxygen therapy has been shown to modulate white cell adherence and be helpful in the initial stages of inflammation. This could be an area of fruitful investigation. In fact, the burn injury may be "the universal trauma model." Wolf and colleagues point out control of the hypermetabolic response and the massive inflammation will be important to improvement in burn care.

Despite the advances of early excision in accelerating healing, we seem to have plateaued in this regard. The authors suggest it may be time to visit the notion of whether healing times can be accelerated. This is another area of the potential benefit of hyperbaric oxygen. A continuing problem in burn therapy and one for future investigation, again suggested by Wolf et al., is the elimination of pain, both acute and chronic. Neuropathic pain is typically difficult to treat and thought to occur in a large percentage of those suffering severe burns.

## Pathophysiology

Physiologic responses to a major burn include a fall in arterial pressure, tachycardia, and a progressive decrease in cardiac output and stroke volume. Metabolic responses are complex and include metabolic acidosis and hyperventilation. Cellular adenosine triphosphate levels fall, resting cell membrane potential decreases, and an intracellular accumulation of sodium, calcium and water is paralleled by a loss of cellular potassium.

Immunologic responses include alteration of macrophage function and perturbation of cellular and humoral immunity [5]. The burn wound is a complex and dynamic injury characterized by a zone of coagulation, surrounded by an area of stasis, and bordered by an area of erythema [6]. The zone of coagulation or complete capillary occlusion may progress by a factor of 10 during the first 48 hours after injury. This phenomenon is three-dimensional; thus, the wound can increase in size and depth during this critical period.

Local microcirculation is compromised to the greatest extent during the 12 to 24 hours post-burn. Burns are in this dynamic state of flux for up to 72 hours after injury [5]. Ischemic necrosis quickly follows. Hematologic changes include platelet microthrombi and hemoconcentration in the post-capillary venules. Edema formation is rapid in the area of injury secondary to increased capillary permeability, decreased oncotic pressure, increased interstitial oncotic pressure, changes in the interstitial space compliance and lymphatic damage [7]. Edema is most prominent in directly involved burned tissues but also develops in distant uninjured tissue, including muscle, intestine and lung. Changes occur in the distant microvasculature, including red cell aggregation, white cell adhesion to venular walls and platelet thromboemboli [8].

Inflammatory mediators are elaborated locally, in part from activated platelets, macrophages and leukocytes. This contributes to the local and systemic hyperpermeability of the microcirculation, appearing histologically as gaps in the venular and capillary endothelium [9]. This progressive process may extend dramatically during the first early days after injury [10-11].

The ongoing tissue damage in thermal injury is due to multiple factors, including the failure of surrounding tis-

sue to supply borderline cells with oxygen and nutrients necessary to sustain viability [6]. Impediment of the circulation below the injury results in dessication of the wound, as fluid cannot be supplied via the thrombosed or obstructed capillaries. Topical agents and dressings may reduce but cannot prevent the dessication of the burn wound and the inexorable progression to deeper layers. Altered permeability is not caused by heat injury alone; oxidants and other mediators (prostaglandins, kinins and histamine) all contribute to vascular permeability [12].

Neutrophils are a major source of oxidants and injury in the ischemia/reperfusion mechanism. This complex may be favorably affected by several interventions. Therapy is focused on the reduction of dermal ischemia, reduction of edema and prevention of infection. During the period of early hemodynamic instability, edema reduction has a markedly beneficial effect as well as modulating later wound conversion from partial- to full-thickness injury [13].

## Infection

Infection remains the leading overall cause of death from burns. Susceptibility to infection is greatly increased due to the loss of the integumentary barrier to bacterial invasion, the ideal substrate present in the burn wound, and the compromised or obstructed microvasculature, which prevents humoral and cellular elements from reaching the injured tissue.

Additionally, the immune system is seriously affected, demonstrating decreased levels of immunoglobulins and serious perturbations of polymorphonuclear leukocyte function (PMNL) [14-15], including disorders of chemotaxis, phagocytosis and diminished killing ability. These altered functions greatly increase morbidity and mortality. Certain patients with specific polymorphisms in the tumor necrosis factor and bacterial recognition genes may have a higher incidence of sepsis than the burn injury alone would predict [16]. More recently, fungal infections have become a therapeutic challenge [17].

Regeneration cannot take place until equilibrium is reached; hence, healing is retarded. Prolongation of the healing process may lead to excessive scarring. Hypertrophic scars are seen in about 4% of cases taking 10 days to heal, 14% of cases taking 14 days or less, 28% of cases taking 21 days to heal, and up to 40% of cases taking longer than 21 days to heal [18].

## Experimental Data

The efficacy of hyperbaric oxygen ($HBO_2$) in the treatment of thermal injury is supported by animal studies and human clinical data. Edema reduction with $HBO_2$ therapy has been demonstrated in burned rabbits[19], rats [20], mice [21] and guinea pigs [22-23]. Improvement in healing time has been reported in burned rabbits [24] and rats [25-26]. Decreased infection rates were an additional observation noted in these models [24-25].

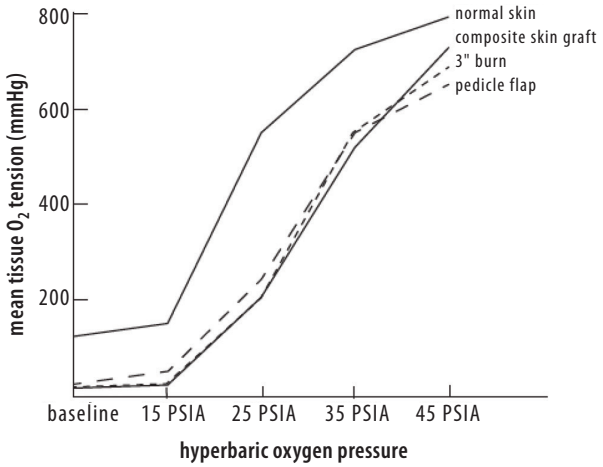
In a seminal study in 1970 Gruber (Figure 1) demonstrated that the area subadjacent to a third-degree burn was hypoxic when compared to normal skin and that the tissue oxygen tension could be raised only by oxygen administered at pressure [27]. Ketchum, in 1967, reported an improvement in healing time and reduced infection in an animal model [24]. He later demonstrated dramatic improvement in the microvasculature of burned rats treated with hyperbaric oxygen therapy [25] (Figure 2).

In 1974 Hartwig [20] confirmed these findings and additionally noted less inflammatory response and suggested hyperbaric oxygen might be a useful adjunct to the technique of early debridement. Wells and Hilton (Figure 3), in a carefully designed and controlled experiment, reported a marked decrease (35%) in extravasation of fluid in 40% of flame-burned dogs [28]. The effect was clearly related to oxygen, and not simply to increased pressure. A reduction in hemoconcentration and improved cardiac output were also noted.

Nylander (Figure 4) [21] in a well-accepted animal model showed that hyperbaric oxygen therapy reduced the generalized edema associated with burn injury.

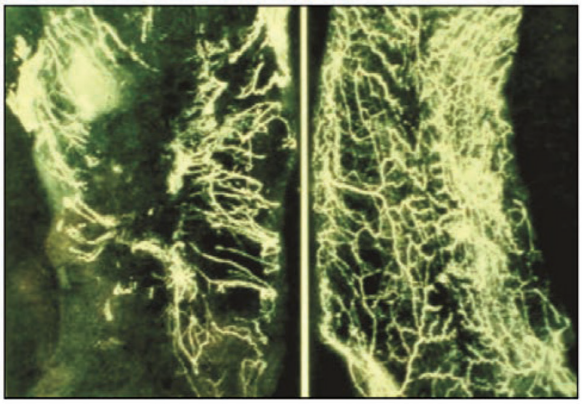
Kaiser (Figure 5) reported that hyperbaric oxygen treatment resulted in shrinkage of third-degree (full-thickness) injury in a rabbit model. Untreated animals demonstrated the expected increase in wound size during the first 48 hours. At all times treated animal wounds remained smaller than those of the controls. A reduction in subcutaneous edema was also observed [22-23]. Stewart and colleagues subjected rats to controlled burn wounds resulting in deep partial-thickness injury. Both experimental groups were treated with topical agents. The hyperbaric oxygen-treated animals showed preservation of dermal elements, no conversion of partial- to full-thickness injury, and preservation of adenosine triphosphate (ATP) levels. The untreated animals demonstrated marked diminution in ATP levels and conversion of partial- to full-thickness injury (Figures 6,7) [29-30].

**Figure 1: Oxygen tension**




Mean oxygen tension of normal skin and various hypoxic tissues as a function of hyperbaric oxygen pressure. **Note:** Oxygen tension rises in burned skin only with increasing pressure. (With permission)

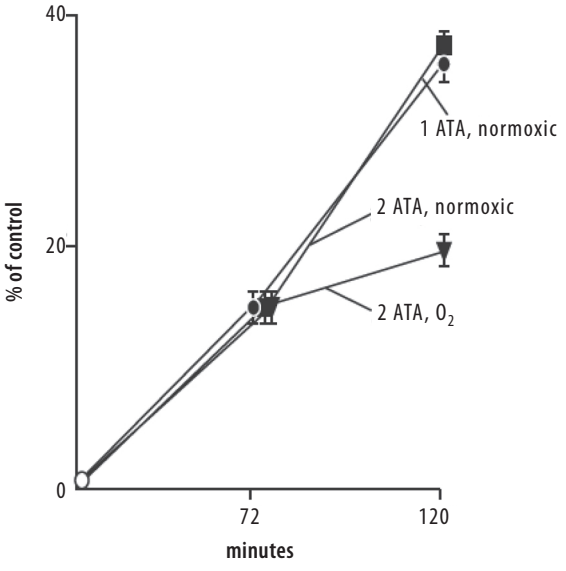
**Figure 2: Capillary state: Control vs. $HBO_2$**



**Left panel:** Capillary disorganization, inflammation and leakage of contrast agent in Control.

**Right panel:** Restoration organized capillary arcades and intact circulation in $HBO_2$-treated animal. (With permission)

**Figure 3: Plasma volume losses**



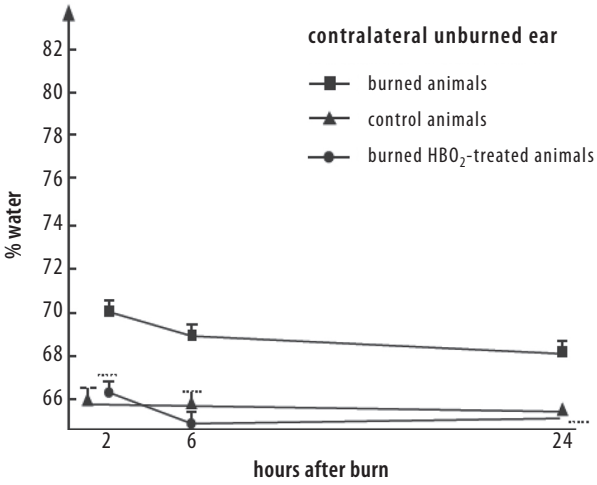

Plasma volume losses after burn in untreated animals (1 ATA, normoxic), animals exposed to hyperbaric oxygen (2 ATA, $O_2$), and to pressure alone (2 ATA, normoxic). (With permission)

These studies may relate directly to the preservation of energy sources for the sodium pump in cellulary physiology. Failure of the sodium pump is felt to be a major factor in the ballooning of the endothelial cells, which occurs after burn injury and subsequent massive fluid losses [10]. Germonpré reported decreased extension of burn injury with $HBO_2$ [31]. $HBO_2$ has also been shown to dramatically improve the microvasculature of burned rats (Hartwig, Ketchum [20,25]). In guinea pigs, earlier return of capillary patency ($p<0.05$) was demonstrated using an India ink technique [32].

Miller and Korn reported faster re-epithelialization ($p<0.001$) from these regenerative sites in guinea pigs treated with $HBO_2$ versus controls. The observed decrease in wound desiccation in the $HBO_2$-treated group was due to preservation of capillary integrity in the zone of stasis [12]. Saunders similarly reported improved dermal circulation, preservation of dermal elements, and less collagen denaturation with $HBO_2$ treatments [33].

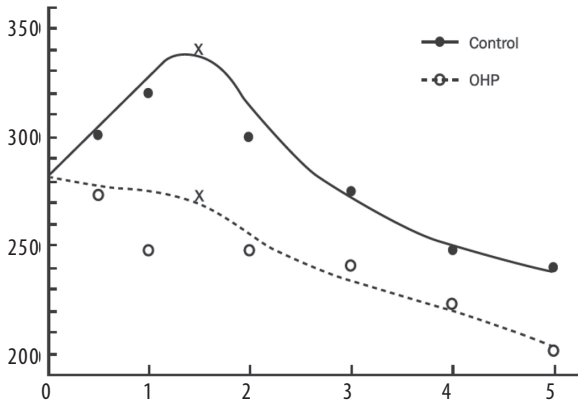
On the other hand, Perrins, in a porcine scald model, failed to demonstrate modification of progressive tissue destruction. However, oxygen was administered at 2 atmospheres absolute (ATA) for only one hour, and treatment occurred over a one-day period only. No vascular studies were undertaken. It was also noted that

**Figure 4: Water content of the unburned contralateral ear**




Water content (+) of the contralateral unburned ear in burned animals with and without $HBO_2$ treatment. (With permission)

**Figure 5: Tissue oxygen tension**




Kaiser and colleagues demonstrated a significant reduction of subcutaneous edema in burned animals treated with $HBO_2$. He reported progression of the burn wound in controls, while in the hyperbaric-treated animals wound size decreased [21]. (With permission)

the porcine model may not be appropriate given the following features about pigs: a natural resistance to skin infection; skin that does not form a blister following scald wound injury; and lack of many shared dermal elements with humans, including cutaneous sweat glands [34].

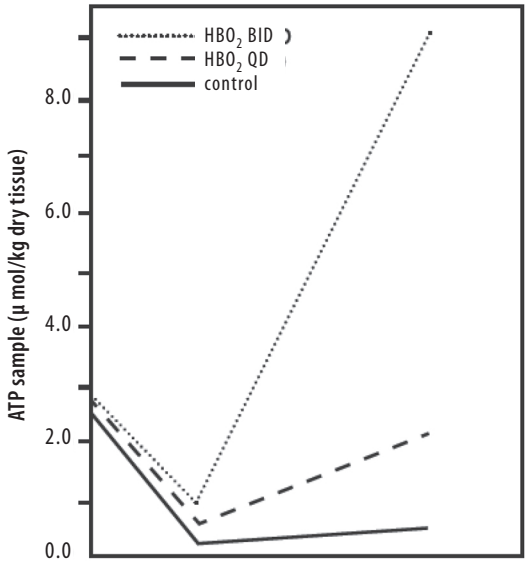
Also highlighting a further study, Niccole reported that $HBO_2$ provided no advantage in the treatment of full-thickness and partial-thickness burns alone or in combination with topical antibiotic therapy in controlling bacterial counts in a rat model. However, despite a treatment delay of 12 hours, hyperbaric oxygen signicantly reduced the time to complete epithelialization in a partial-thickness burn injury [26].

The pathophysiologic changes within the burn wound show a striking similarity to those noted in the ischemia reperfusion injury, i.e., depletion of ATP, production of xanthine oxidase, lipid peroxidation, activation of polymorphonuclear cells with subsequent endothelial adherence and generation of reactive oxygen species (ROS) [35-38].

Recent data regarding $HBO_2$ cardiac preconditioning (inducing cellular tolerance and protection from ischemia) and adaptive responses resulting in cardioprotection and attenuation of ischemia-reperfusion injury are mediated by $HBO_2$-induced reactive oxygen species (ROS) (e.g., superoxide and hydrogen peroxide) that stimulate the production of nitric oxide. $HBO_2$-induced ROS are known to initiate gene expression and reduce neutrophil adhesion (via a decrease in CDl1a/18 function, P-selectin and downregulation of intracellular adhesion molecule-1). $HBO_2$ also decreases lipid peroxidation, stimulates neovascularization and increases antioxidants, thus resulting in cardioprotection [39]. Elucidation of these mechanisms for cardioprotection may provide further understanding of the mechanisms whereby hyperbaric oxygen is of benefit in acute thermal injury.

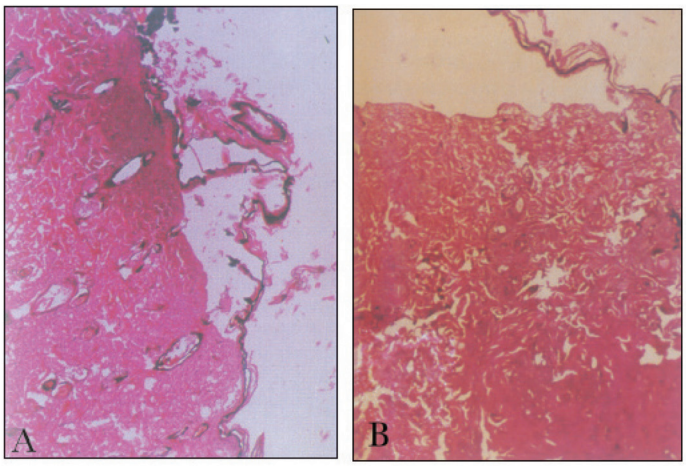
In a model of reperfusion injury, Zamboni demonstrated that hyperbaric oxygen is a potent blocker of white cell adherence to endothelial cell walls in skeletal muscle, interrupting the cascade that causes vascular damage [40]. The mechanism is felt to be an inhibitory effect on the CD18 locus [41]. As discussed by Wasiak et al. [42], inhibition of beta 2-integrin activation of intracellular adhesion molecule one (ICAM-1) [43] enables tissues to maintain microvascular flow in

**Figure 6: Rat burns treated with sulfadiazine dressing**




(With permission)

**Figure 7: Partial-thickness burns**




Biopsy of experimental partial-thickness burns at five days.
A. Left: $HBO_2$-treated animals show preservation of the dermal elements.
B: Right: Non-treated animals show coagulation necrosis.
(With permission)

areas otherwise subject to the well-described "secondary injury" following a thermal burn [8]. This effect persists for some hours, as demonstrated by both Ueno [44] and Milijkovic-Lolic [45]; Germonpré's data support this observation and may explain the beneficial effect of hyperbaric oxygen therapy on the microcirculation previously observed [20,29-31,33].

Shoshani reported no benefit of $HBO_2$ in a rat burn model where all animals received standard sulfadiazine treatment [46]. There was no difference in burn wound size, re-epithelialization rate, Doppler blood flow or healing. In this report, the author erroneously stated that this was the first study utilizing standard burn care (topical agents). Compared to the earlier study by Stewart's group, which utilized silver sulfadiazine dressings and confirmed preservation of dermal elements [29-31], these contradictory findings might be explained by methodological differences.

Bleser and Benichoux, in a very large controlled study in a rat model of 30% body surface area (BSA) burns, reported reduced burn shock and a fourfold increased survival in $HBO_2$-treated animals versus controls [47]. Tenenhaus and colleagues showed reduction in mesenteric bacterial colonization ($p<0.005$) in an $HBO_2$-treated burned mouse model [48]. Bacterial translocation is felt to be a major source of burn wound infection.

In 2005 Magnotti et al. proposed an evolution from bacterial translocation to gut ischemia-reperfusion injury after burn injury as the pathogenesis of multiple organ dysfunction syndrome. Systemic inflammation, acute lung injury and multiple organ failure after a major thermal injury are relatively common causes of morbidity and mortality. In the normal (unburned) host, the intestinal mucosa functions as a major local defense barrier, a component of multiple defense mechanisms that helps prevent gut bacteria, as well as their products, from crossing the mucosal barrier. After a major thermal injury, and in other clinical and experimental situations, this intestinal barrier function becomes overwhelmed or impaired, resulting in the movement of bacteria and/or endotoxin to the mesenteric lymph nodes and systemic tissues, defined as bacterial translocation. The importance of this intestinal barrier function becomes clear when considering that the distal small bowel and colon contain $10^{10}$ concentrations of anaerobes and $10^5$ to $10^8$ each of Gram-positive and Gram-negative aerobic and facultative microorganisms per gram of tissue, and enough endotoxin to kill the host thousands of times over [49].

Loss of gut barrier function and a resultant gut inflammatory response lead to the production of proinflammatory factors; this can cause a septic state, leading to distant organ failure. Splanchnic hypoperfusion leading to gut ischemia-reperfusion injury appears to be the dominant hemodynamic event, triggering the release of biologically active factors into the mesenteric lymphatics. The benefits of the early use of hyperbaric oxygen in burn victims may in part be mediated through amelioration of gut reperfusion injury. The beneficial effects of $HBO_2$ in ischemic-reperfused tissues have been demonstrated in intestine [50], skeletal muscle [40,51] brain [52-54] and testicular tissue [55], and myocardium [39,56-58]. In a study of severely burned humans (>30% TBSA), $HBO_2$-treated patients compared to controls had increased levels of serum-soluble interleukin-2 receptor ($p<0.05$) and decreased plasma fibronectin ($p<0.01$), resulting clinically in a lower incidence of sepsis ($p<0.05$) [59].

Total enteral nutrition, starting as early as possible after thermal injury, is recommended for burn patients. It results in decreased morbidity and mortality, and supports intestinal structure and function. Studies of intestinal barrier function biology, pathophysiology and consequences of gut barrier failure demonstrate that the ischemic and/or stressed gut can become a proinflammatory organ [60], and gut-derived factors liberated after periods of splanchnic hypoperfusion can lead to acute distant organ, cellular dysfunction and activation of neutrophils and other proinflammatory cells [61].

Reduction of PMNL-killing ability in hypoxic tissue has been well documented [62-63]. The ability of hyperbaric oxygen to elevate tissue oxygen tension and the enhancement of PMNL killing in an oxygen-enriched animal model as demonstrated by Mader [63] suggest that this may be an additional benefit of $HBO_2$. Hussman and colleagues have shown no evidence of $HBO_2$-induced immunosuppression in a carefully controlled animal model [65].

In a 2005 randomized controlled study Bilic evaluated the effects of $HBO_2$ on burn wound healing. Standard deep second-degree burns were produced in male Wistar rats treated with silver sulfadiazine and then randomly assigned to either a normoxic, placebo gas or to 2.5 ATA $HBO_2$ for 60 minutes for a total of 21 sessions. $HBO_2$ had a beneficial effect on post-burn edema ($p=0.022$), neoangiogenesis ($p=0.009$), numbers of regenerative active follicles ($p=0.009$), and time to epithelial regeneration ($p=0.048$). There were no significant differences in necrosis staging or margination of leukocytes. The authors concluded that the data support earlier conclusions that $HBO_2$ is of benefit in the healing of burn wounds [66].

Turkaslan et al. [67] reported that hyperbaric oxygen treatment reduced progression of the zone of stasis in the first 24 hours after injury and accelerated the healing process by supporting neoangiogenesis. Prevention of progression in the zone of stasis is a major goal in burn therapy. This report lends further credence to the previously cited work of Miller, Korn, Hartwig and Ketchum.

$HBO_2$ has been shown to mobilize stem/progenitor cells in both humans and mice by stimulating bone marrow stromal cell type 3 (endothelial) nitric oxide synthase [68-72]. Findings indicate that some of the mobilized cells will home to peripheral sites where they function as de novo endothelial progenitor cells (EPCs), contributing to wound vasculogenesis, a complement to local angiogenesis. Additionally, at peripheral sites $HBO_2$ stimulates stem cell growth and differentiation by engaging a physiological autocrine loop responsive to oxidative stress, much the same as lactate [73-75].

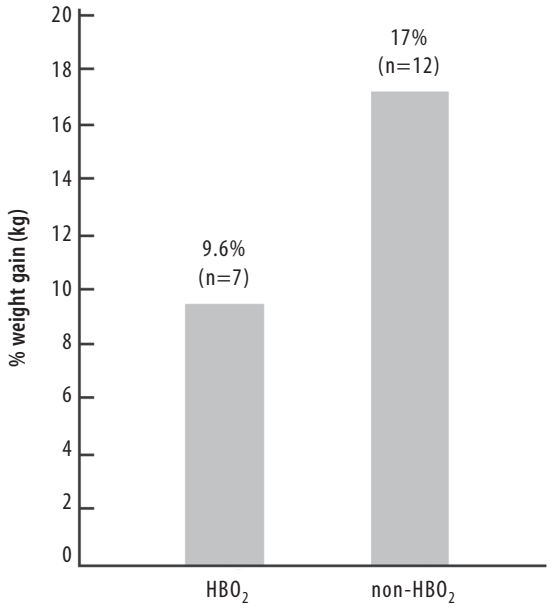
$HBO_2$ stimulates peripheral site EPCs recruitment and differentiation via a pathway involving thioredoxin-1, hypoxia-inducible factors-1 (HIF-1) and HIF-2. These findings provide new insight into possible mechanisms for the known clinical benefits of hyperbaric oxygen.

The overwhelming body of evidence in a large number of controlled animal studies demonstrates that hyperbaric oxygen reduces dermal ischemia, reduces edema, prevents conversion of partial- to full-thickness injury, preserves the microcirculation, and preserves ATP and cellular integrity. Additional benefits may be enhancement of PMNL killing and modulation of ischemia reperfusion injury, resulting in improved survival.

## Clinical experience

In 1965, Wada observed improved healing of burns in coal miners being treated for carbon monoxide poisoning with $HBO_2$. Later clinical series by Ikeda, Wada, Lamy, Tabor and Grossman [19,75-80] showed improved healing [75], decreased length of hospital stay [80], decreased mortality [80-81], decreased overall cost of care [80-82] improved morbidity [80], decreased fluid requirements (30-35%),81 and decreased number of surgeries ($p<0.041$) [82]. Niu reported a very large clinical outcome series showing a statistically significant reduction in mortality ($p=0.028$) in 266 seriously burned

**Figure 8: Maximum weight gain / 3 days**




Maximum weight gain at three days expressed as percentage of admission weight. $HBO_2$-treated patients showed a 45 percent reduction in weight gain ($p<0.03$) [87]. (With permission)

patients who received $HBO_2$ when compared to 609 control patients [81]. The author also observed a lower incidence of infection and stated that $HBO_2$ allowed the burn surgeon more time to more accurately define the extent of injury.

Cianci has shown a significant reduction in length of hospital stay in burns up to 39% TBSA [83]. Additionally noted was a reduction in the need for surgery, which included grafting, in a series of patients with 40-80% burns when compared to non-$HBO_2$-treated controls. $HBO_2$-treated patients showed an average savings of 36% ($120,000) per case [82]. Adjusted for inflation, this would represent a saving of $227,000 per case in 2016 U.S. dollars.

Hart reported a sham controlled randomized series showing reduced fluid requirements, mean healing time ($p<0.005$), mortality and morbidity in 10-50% TBSA burn patients treated with $HBO_2$ when compared to controls and to United States National Burn Information Exchange Standards [85].

Frequently cited as a negative study, in a retrospective paired controlled series of burn patients treated with $HBO_2$ Waisbren reported increased sepsis, reduced renal function and decreased circulating white blood cells in $HBO_2$-treated patients. The author stated he could demonstrate neither a salutory nor deleterious effect on mortality [86]. Despite these negative conclusions, it should be noted that there was an important 75% reduction in the need for grafting ($p<0.001$) in the hyperbaric group.

In a randomized controlled study of 37 partial-thickness burn patients treated with $HBO_2$ versus 37 controls, Merola reported increased granulation, faster healing and decreased scarring [87].

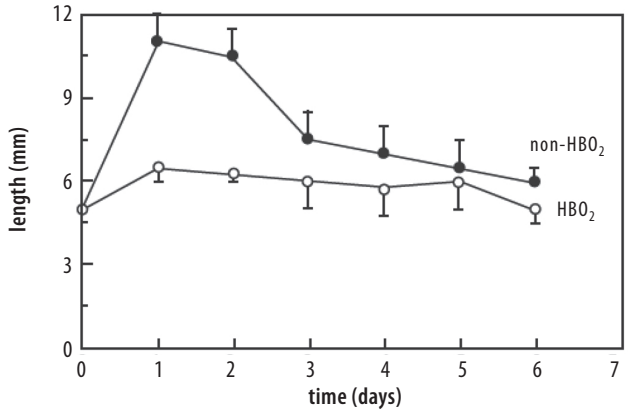
Cianci observed similar results in a series of patients averaging 28% TBSA burns [88]. In a small blinded review, Cianci's group reported a 25% reduction in resuscitative fluid requirements ($p<0.07$) and maximum (and percent) weight gain ($p<0.012$) in seriously burned (40-80% TBSA) patients treated with adjunctive $HBO_2$ versus controls at a regional burn center [82,84] (Figure 8) [89].

In a controlled pilot series, Maxwell reported reduced surgery, resuscitative weight gain, intensive care days, total hospitalization time, wound sepsis and cost of hospitalization in the $HBO_2$ group [90]. Cianci reported reduced surgeries ($p<0.03$), length of hospital stay (53%) and cost of care (49%) in 40-80% TBSA burns [91]. Hammarlund and colleagues showed reduced edema and wound exudation in a controlled series of human volunteers with UV-irradiated blister wounds [92] (Figure 9).

In a subsequent similar study, Niezgoda (Figure 10) demonstrated reduced wound size ($p<0.03$), laser Doppler-measured hyperemia ($p<0.05$) and wound exudate ($p<0.04$) in the $HBO_2$-treated group. This study was the first prospective randomized, controlled, double-blinded trial comparing $HBO_2$ with sham controls in a human burn model [93].

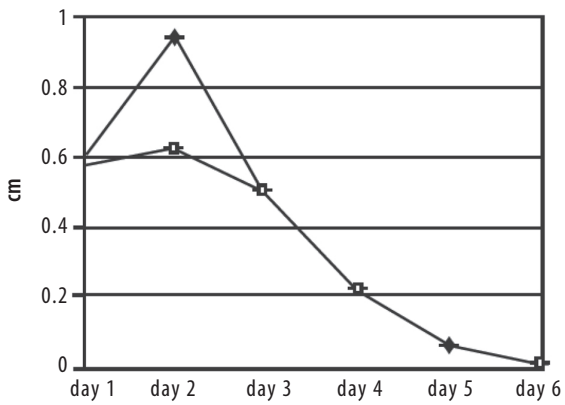
In a study purporting to conclude limited positive impact of hyperbaric oxygen therapy for burn patients, Brannen et al. in 1997 [94] reported a randomized prospective trial of hyperbaric oxygen in the treatment of burn injury. Sixty-three patients received hyperbaric oxygen, and 62 served as controls. One-third of the hyperbaric-treated patients received their first treatment within eight hours of injury. However, the average time to treatment was 11.5 hours after the burn injury. The authors noted no difference in the outcome measures of mortality, number of operations, or length of stay, stating they were unable to demonstrate any significant benefit to burn patients from the use of $HBO_2$.

**Figure 9: Maximum length as function of time**




Maximum length (including edema adjacent to the wound) (mean ± s.d.) of UV-irradiated (•) and $HBO_2$-treated UV-irradiated (○) blister wounds as a function of time. The value on (○) is approximately the diameter of the suction cup used to create the blister ($p<0.05$). (With permission)

**Figure 10: Hyperbaric oxygen therapy for burns**




Wound size measurements (cm) of UV-irradiated suction blister wounds in control group (❑) and hyperbaric oxygen group (✦). Graph courtesy of Dr. Jeffrey A. Niezgoda.

There were serious limitations in this study. Two-thirds of patients did not receive their first treatment until more than eight hours after burn injury, with a mean of 11.5 hours. Results in the subset of patients receiving earlier treatment were not examined separately. Important outcome measures not studied were functional and cosmetic aspects of facial, hand and perineal burns. Length of stay, number of surgeries, and extent of grafting are subject to a variety of confounding influences, including economic (e.g., hospital and insurance, utilization management, physician reimbursement) and social considerations (e.g., lack of adequate housing, caregivers and rehabilitation efforts).

Despite randomization for age, burn size and inhalation injury, the populations were still heterogeneous. Comorbidity was not examined. Further, all patients underwent exceedingly early and aggressive excisional therapy, with rapid discharge to a lesser level of care. While the authors failed to conclude there were significant benefits of $HBO_2$ for burn patients in this study, the authors did observe less fluid loss, drier wounds that necessitated fewer dressing changes, and earlier healing. Further analysis also showed a significant reduction in hospital costs in the hyperbaric group.

## Recent perspectives

### *Pain management*

There is a substantial body of evidence reporting the use of hyperbaric oxygen therapy in pain attenuation. Sutherland and colleagues [95] have done an excellent review of the literature and conclude that hyperbaric oxygen therapy has been proven to demonstrate a significant antinociceptive effect. They state that early clinical research indicates that hyperbaric oxygen therapy may be useful in modulating human pain; however, further studies are required to determine whether $HBO_2$ is a safe and efficacious treatment modality.

A particularly difficult problem for some burn patients is that opioid overuse contributes to adaptive immune suppression, and this may be associated with poorer outcomes. Neuropathic pain is typically difficult to treat and is thought to occur in a large percentage of those suffering severe burns. Rasmussen and colleagues [96] reported a series of 17 patients who underwent a controlled first-degree burn. One group was treated at atmospheric pressure with a fraction of inspired oxygen ($FiO_2$)=0.21 during hyperbaric treatment. Another group was treated at

2.4 ATA breathing 100% $O_2$. Patients who underwent chamber treatment demonstrated attenuation of secondary hyperalgesia, i.e., an antinociceptive effect. The authors state that post-burn hyperbaric oxygen therapy has a potent antinociceptive effect that works at a central desensitization level. The authors suggest this thermal injury model may give impetus to future neurophysiologic studies exploring the central effects of hyperbaric oxygen treatment.

Chong et al. [97] reported a group of 17 burn patients who were treated with hyperbaric oxygen or routine burn care. They noted no difference in inflammatory cytokines or depth of burns, though patients in both groups either increased or reduced estimated depth of injury. There were fewer positive biopsies for bacterial colonization in the hyperbaric group. They also related that this was a preliminary study and of insufficient power to determine any real statistical significance, as 40 patients would have been required to achieve this goal. It was unclear as to the average time from injury to the provision of hyperbaric oxygen therapy. However, it was stated that patients were treated during "routine hyperbaric treatment sessions" and that the $HBO_2$ patients received two treatments within the first 22 hours. It would be more appropriate to treat as soon as the patient is stable, as reported by others.

Jones et al. [98] reported a series of diabetic patients suffering foot burns. Transcutaneous $O_2$ studies were performed, and those patients who had low $TcO_2$s and responded to an oxygen challenge underwent hyperbaric oxygen therapy in addition to standard care. There were 18 patients in the hyperbaric study group. All healed, with one amputation. The authors compared this to a group of 68 patients treated with traditional care. Eleven patients in this cohort suffered 31 amputations. The authors reported the observations of their burn surgeons that, with a larger sample size, a definite benefit could be demonstrated. Of note, three of the patients who were scheduled for grafting healed spontaneously with $HBO_2$ alone.

### *Immunity and infection*

A major and continuing clinical problem is the innate inflammatory response induced by genetic factors such as those common in neutrophils and macrophages, which are massively increased while the T-cell adaptive responses are downregulated. Zhang and colleagues [99] have shown that hyperbaric oxygen attenuates apoptosis and decreases inflammation in an ischemic wound model. The effect of hyperbaric oxygen on modulation of white cell adherence to endothelium has been described. Thom et al. studied the effect of hyperbaric oxygen and demonstrated that $HBO_2$ additionally does not alter platelet function but inhibits Beta 2-integrin adhesion to endothelium at pressures of 2.8 or more. This would have a beneficial effect on the early stages of burn injury [100].

### *Stem cell effects*

Thom et al. reported that hyperbaric oxygen increases marrow stem cell populations, and these cells migrate to areas of wounding [101-102]. The previously reported preservation of dermal elements, specifically, hair follicles, may represent an additional area for recruitment of native stem cells in the healing of burns. This was described by Stewart et al. [103-104]. These should be areas of fruitful research in burn patients where prolonged healing is a major problem.

### *Antioxidant effects*

Concern about oxygen toxicity is valid. However, Sureda and colleagues [105] studied the effect of hyperbaric oxygen therapy in chronic wounds and reported that this modality actually enhanced plasma antioxidant defenses and contributed to the activation of healing resolution, angiogenesis, and vascular tone regulation by increasing the vascular endothelial growth factor (VEGF) and interleukin-6 release and the endothelin-1 decrease. These may be significant factors in simulating wound healing. In clinical practice, acute oxygen toxicity is very rare and usually associated with prolonged treatments utilized in decompression sickness.

## Inhalation injury

Considerable attention has been given to the use of $HBO_2$ in inhalation injury due in part to fear that $HBO_2$ may cause worsening of pulmonary damage, particularly in those patients maintained on high levels of inspired oxygen. The more extensive the burn injury, the higher the incidence of an inhalation injury [106]. Pulmonary injury caused by smoke inhalation is a major cause of fire-related deaths [107]. The airway injury can be worsened by a variety of chemical pyrolysis products, depending on the material burned [108].

Grim studied products of lipid peroxidation in the exhaled gases in $HBO_2$-treated burn patients and found no indication of oxidative stress [109]. In comparison with a comparable size burn alone, the combination of

a body burn and smoke inhalation injury results in a marked increase in mortality and morbidity, in hemodynamic instability, in burn wound edema, a 30-50% increase in initial fluid requirements and an accentuation of lung dysfunction.

Ray analyzed a series of severely burned patients being treated for concurrent inhalation injury, thermal injury and adult respiratory distress syndrome [110]. The author noted no deleterious effect of $HBO_2$, even in those on continuous high levels of inspired oxygen. More rapid weaning from mechanical ventilation was possible in the $HBO_2$-treated group (5.3 days vs. 26 days, $p<0.05$). There was a significant reduction in cost of care per case of $67,000 in the $HBO_2$-treated patients ($p<0.05$). Adjusted to 2016 U.S. dollars, this figure would be $121,000. There is no current evidence to controvert these studies.

### 2009 Cochrane Review

In a 2009 Cochrane Database systemic review of the efficacy of $HBO_2$ for thermal burns, Villanueva et al. identified four randomized controlled studies, of which two satisfied their inclusion criteria [111].

In the first trial in 1974 [85] Hart reported reduced fluid requirements and mean healing time ($p<0.005$), and reduced mortality and morbidity when compared to controls. There was also a reduction in mortality and morbidity when compared to the National Burn Information Exchange standards.

Because of heterogeneity, the studies could not be pooled, although Hart reported mean healing time as significantly shorter (19.7 vs. 43.8 days ($p<0.001$)). The authors suggested that the Hart study was particularly constrained by lack of power to detect useful clinical differences. The Brannen study [94], reporting no difference in mortality, length of stay or surgeries, was constrained by the previously mentioned limitations. The authors state that while there are promising results from non-random clinical reports, there is insufficient evidence to recommend or refute the routine use of hyperbaric oxygen for the treatment of thermal burns. The authors further suggest that a large multicenter randomized study of sufficient power would be needed to address these shortcomings. The Cochrane report did not consider several outcome studies with matched controls showing reduced length of stay, reduction in fluid requirements and edema, reduction of surgery and cost effectiveness.

While these reports certainly had limitations, they represent valid analysis of the benefits of early treatment in thermal injury and underscore that the observations of skilled and experienced physicians remain an important component of determining therapeutic efficacy. A well-designed, randomized, blinded control study with sham treatment and sufficient power is certainly desirable yet remains to be performed. Most centers see very few large burns; only 4% of burn admissions are for burns >40% TBSA, certainly necessitating a multicenter format. Attempts at organizing such a study have so far been unsuccessful.

## Clinical management

### *Surgical perspectives*

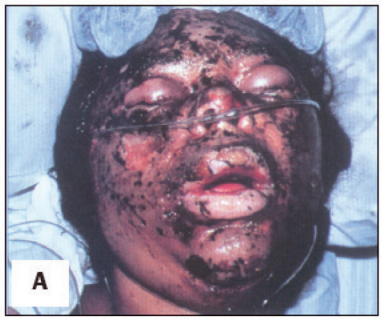
Over the past 40 years, the pendulum has swung to an aggressive surgical management of the burn wound, i.e., early tangential or sequential excision and grafting of the deep second-degree and probable third-degree burns, especially to functionally important parts of the body [112-116]. Hyperbaric oxygen, as an adjunctive therapy, has allowed the surgeon yet another modality of treatment for these deep second-degree burns, especially including those to the hands and fingers, face and ears, and other areas where the surgical technique of excision is often imprecise and coverage is sometimes difficult.

These wounds, not obvious third-degree, are then best treated with topical antimicrobial agents, bedside and enzymatic debridement, wound care, including biological dressings, and adjunctive hyperbaric oxygen therapy, allowing the surgeon more time for healing to take place and for definition of the extent and depth of injury (Figures 11-13).

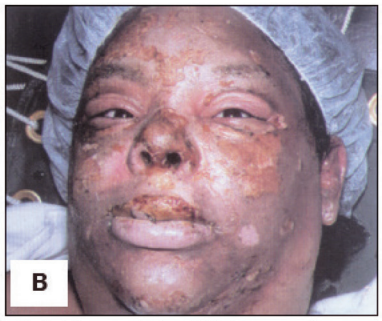
Adjunctive hyperbaric oxygen therapy has drastically reduced the healing time in the major burn injury, especially if the wounds are deep second-degree [81-83,88]. There is theoretical benefit of $HBO_2$ therapy for obviously less well-defined third-degree burns [23]. Fourth-degree burns, most commonly seen in high-voltage electrical injuries [117], are benefited by reduction in fascial compartment pressures, as injured muscle swelling is lessened by preservation of aerobic glycolysis and, later, by a reduction of anaerobic infection.

Finally, reconstruction utilizing flaps, full-thickness skin and composite grafts, i.e., ear-to-nose grafts, has been greatly facilitated using adjunctive $HBO_2$ [121]. Often the decision to use $HBO_2$ therapy has been made intraoperatively when a surgeon is concerned about a compromised cutaneous or myocutaneous flap. Patients are, in many instances, prepared preoperatively about the possibility of receiving adjunctive $HBO_2$ therapy immediately postoperatively.

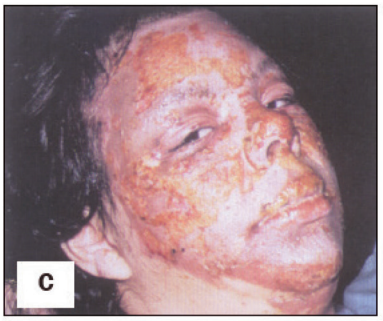
**Figure 11: Burn victim's recovery**



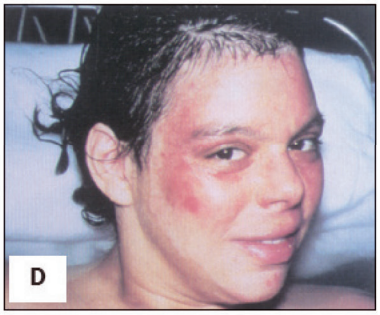

**A.** 23-year-old white female with facial burns from flaming gasoline and tar 12 hours after injury.




**B.** 24 hours later (36 hours after injury) after two $HBO_2$ treatments. Note resolution of edema.




**C.** 72 hours later (84 hours after injury) after six $HBO_2$ treatments.




**D.** Shortly before discharge.


**E.** Four years after discharge.

Note: Consent to use these photos was obtained prior to publication.

Units planning treatment of burn patients should be experienced in management of critical-care patients in the hyperbaric setting and specific problems of burn patients prior to initiation of a therapy program. Preferably, personnel should be certified in burn care and hyperbaric oxygen therapy. The hyperbaric department should function as an extension of the burn unit and participate in the team approach to burn management.

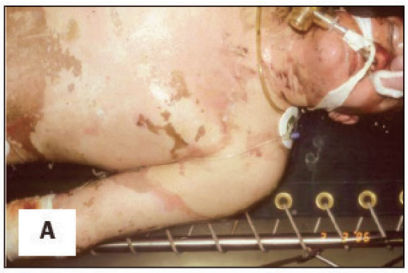
### *Hyperbaric oxygen*

Hyperbaric oxygen therapy is begun as soon as possible after injury, often during initial resuscitation. Treatments are attempted three times within the first 24 hours and twice daily thereafter on a regimen of 90 minutes of 100% oxygen delivery at 2.0 to 2.4 ATA. Early experience in treating children recommends 45 minutes twice daily [78], but more recent extensive clinical use of $HBO_2$ in children demonstrates that adult protocols are safe.

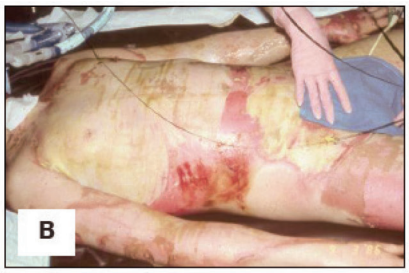
Patients are monitored during initial treatment and as necessary thereafter. Blood pressure can be monitored via transducers or non-invasively using blood pressure cuffs designed for use in monoplace chambers. Patients can be maintained on ventilator support during treatment, which is frequently the case in larger burns with concurrent inhalation injury.

Careful attention to fluid management is mandatory. Initial requirements may be several liters per hour, and pumps capable of this delivery at pressure must be utilized in order to maintain appropriate fluid replacement in the hyperbaric chamber. In larger burn injuries, adequate fluid and electrolyte resuscitation during the first 24 hours can be problematic. Certain patients can develop hypotension shortly after exiting the chamber. Careful volume replacement and assessment of fluid status is mandatory prior to, during, and immediately after $HBO_2$ treatment. Increasing fluids during ascent may help compensate for any hypovolemia unmasked after hyperbaric oxygen exposure.

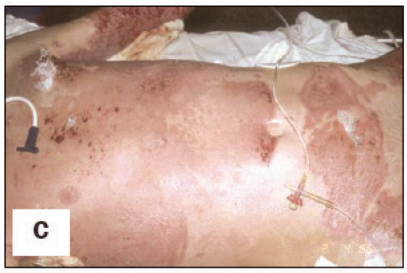
**Figure 11: Burn victim's recovery**




**A.** 18-year-old white male with deep partial- to full-thickness burns from flame burn, TBSA 70%. Photo taken before $HBO_2$.

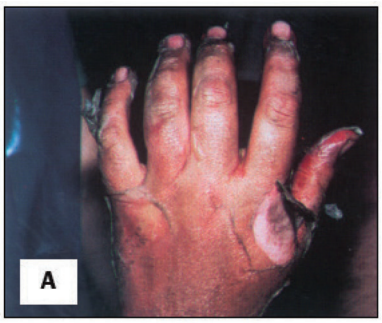



**B.** Patient six days later after $HBO_2$ twice daily.

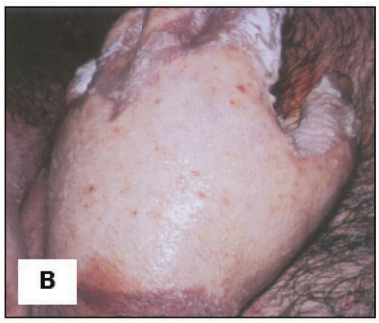



**C.** 30 days later with $HBO_2$. No skin grafts required on chest and torso.

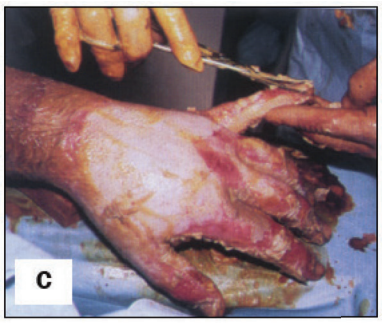
**Figure 11: Burn victim's recovery**



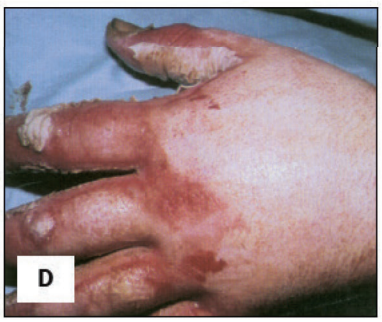

**A.** Deep partial-thickness burn to hand of 30-year-old white male with burn, TBSA 60% and inhalation injury Photo taken on admission.



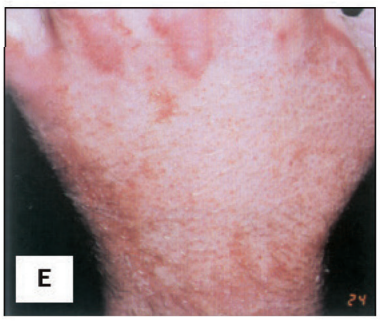

**B.** Patient six days later.



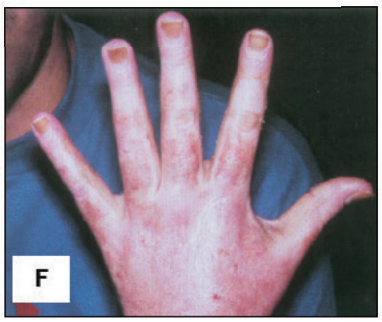

**C.** At surgery, light debridement.




**D.** Immediately after surgery. Note preservation of dermal appendages.




**E.** Two weeks after admission. Note re-epithelialization.




**F.** Appearance on discharge 25 days post-injury, Healed without grafting.

Note: Consent to use all photos on this page was obtained prior to publication.

Maintenance of a comfortable ambient temperature must be accomplished. Thermal instability may be a problem within one to two hours of burn wound cleansing and dressing change (depending on the methods used), especially in large TBSA burns. These patients should be carefully assessed prior to an $HBO_2$ exposure. Febrile patients must be closely monitored and fever controlled, as oxygen toxicity is reported to be more common in this group.

In large burns of 40% TBSA or greater, treatment is rendered for 10 to 14 days in close consultation with the burn surgeon. Many partial-thickness burns will heal without surgery during this time frame and obviate the need for grafting. Treatment beyond 20-30 sessions is usually utilized to optimize graft take. While there is no absolute limit to the total number of hyperbaric treatments, it is rare to exceed 40 to 50 sessions, and utilization review is recommended.

Concern has been expressed about the use of the carbonic anhydrase inhibitor mafenide acetate (Sulfamylon) and its removal recommended prior to $HBO_2$ treatment based on the potential for $CO_2$ buildup, which can lead to vasodilatation [122]. Sulfamylon is less frequently utilized in burn centers, and rarely used at the authors' facility except in select cases (small TBSA, severe infection and/or contraindication to silver sulfadiazine). Its limited use in this setting has not resulted in any observed untoward effects [123]. Silver sulfadiazine is the most widely used topical therapy because of its relatively low toxicity and ease of use [9].

In larger TBSA burns, especially of the head and neck, otic barotrauma may be a problem, and careful attention should be given to this potential complication. The $HBO_2$ team should make use of early ENT consultation when indicated.

Patients may be treated in a multiplace or monoplace configuration. Movement over long distances is not recommended; therefore patients should not be transported to a hyperbaric chamber that is not within the same facility as the burn center [124].

### Patient selection criteria

Hyperbaric oxygen therapy is recommended to treat serious burns, i.e., greater than 20% total body surface area, and/or with involvement of the hands, face, feet or perineum that are deep partial- or full-thickness injury. Patients with superficial burns or those not expected to survive are not accepted for therapy. Transfer of patients for $HBO_2$ treatment should be considered carefully and should be sent only to a facility that has both a hyperbaric chamber and a burn unit.

### Utilization review

Utilization review is recommended after 30 hyperbaric oxygen sessions.

### Cost impact

Burn care is expensive. During 1997-98, in a Northern California regional burn center (Doctors Medical Center Burn Center), hospital costs for 20 burn patients averaged $253,000 ($393,000 in 2016 U.S. dollars) [125]. This includes the cost of hyperbaric oxygen that averaged $6,360 ($10,000 in 2016 U.S. dollars) per patient.

Cost data from the 2012 National Burn Repository report indicate that for patients who survive 60% total body surface area burns, charges average $1,297,000,000 (in 2016 U.S. dollars) for the hospital stay alone. This does not include operating room time, surgeon's bills, artificial skin, rehabilitation and other costs that can reach $637,000 in 2016 U.S. dollars or more for burns over 80% TBSA [1].

Although not calculated, cost savings as a result of the use of $HBO_2$ in acute thermal injury are implied in all of the 22 clinical studies in this report by demonstrating reductions in healing time, hospital length of stay, and numbers of surgeries including grafting. In six of the studies, the authors specifically analyzed costs of care in thermal injury with and without adjunctive $HBO_2$, estimating a range of average savings in patients treated with $HBO_2$ from $76,000 to $120,000 per case.

## DISCUSSION

Despite the many advances in burn therapy, including early excision, nutritional support, improved ventilation and infection control, since the mid-1980s there appears to have been little change in mortality except for patients over 65 with larger burns. Early excision seems to have decreased mortality and overall length of stay in smaller burns and for patients not suffering concurrent inhalation injury [126]. Engrav, in a review of 35 years' experience at the Harborview Burn Center in Seattle, Washington, reported that early excision did not decrease length of stay for larger burns, with little change since 1990 [127]. It has also been suggested that burn care may have already achieved "a floor of survival" [128]. Thus, further improvement in burn care, length

of stay, mortality and cost containment must be addressed by additional therapeutic developments. Adjunctive hyperbaric oxygen therapy has been shown to reduce length of stay and cost of care in conjunction with early excision and comprehensive burn management. The reader is directed to a comprehensive and recent review on priorities of burn research [129].

## SUMMARY

Hyperbaric oxygen in the treatment of burns has been demonstrated in numerous animal studies and human reports over the last 40 years. Observations utilizing hyperbaric oxygen therapy after burns have shown reversal of the zone of stasis, reduction of ischemia and ischemic necrosis, prevention of progression of partial- to full-thickness injury, moderation of inflammation, lessening of the capillary leak, preservation of dermal elements, a reduced need for grafting, shortened hospital stay, and a reduction in cost of care.

The burn community has pointed out the need for improvement in our control of pain, speed of healing, and scarring. Wolf and Engrav have both reported limited progress in burn therapy that has been made in the last 20 years, especially in the control of the inflammatory state, the hypermetabolic syndrome, infection, and scarring. Perhaps the time has come for the burn community to routinely consider hyperbaric oxygen in the treatment of clinical burns.

"It is clear from review of collected research and clinical data that hyperbaric oxygen therapy provides a unique environment for wound recovery and tissue regeneration for thermal injury that cannot be comparably achieved by our current surgical and medical therapies. Evidence of the reduction in patient morbidity and length of hospital stay has been observed in the majority of clinical observations regarding the use of $HBO_2$ therapy in burn management and should be expected to be gained from carefully structured programs utilizing these methods. The scientific evidence of the efficacy of $HBO_2$ therapy as an effective tool for wound healing has made exceptional gains over the past three decades and provides us with a firm biological and physiologic basis for the use of this therapy in patients with complex wounds and burns. The scientific gains made from the observations of $HBO_2$ therapy-related mechanisms for stem progenitor cell signaling and wound healing have also been significant. Finally, research documenting the vulnerary effects of $HBO_2$ therapy at the cellular and molecular levels also suggests that this therapy has the potential to provide a much-needed elevation of the "floor of survival" for burn victims and should provide for substantial enhancements in their wound healing and quality of life" [130].

Additional clinical evidence for the efficacy of hyperbaric oxygen therapy in burns would ideally result from a well-designed, multicenter, randomized study of sufficient power. While we await more data [129], we should remember that the observations of seasoned clinicians also remain a valid test of efficacy.

Current data show that hyperbaric oxygen therapy, when used as an adjunct in a comprehensive program of burn care, is a cost-effective modality that can significantly improve morbidity and mortality, reduce length of hospital stay, and lessen the need for surgery. It has been demonstrated to be safe in the hands of those thoroughly trained in rendering hyperbaric oxygen therapy in the critical-care setting and with appropriate monitoring precautions. Careful patient selection and screening are mandatory.

Given our current understanding of the uniquely beneficial effects of hyperbaric oxygenation on the cellular and molecular mechanisms of wound healing, it is suggested that the formal integration of hyperbaric oxygen therapy in early burn wound management be thoughtfully considered, as well as further investigated in well-designed multicenter studies that may provide data for burn wound healing and burn patient outcomes supportive of this role.

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### *Conflict of interest statement*

*The authors declare no conflicts of interest.*

## REFERENCES


1. American Burn Association. National burn repository. Version 8.0. 2012. Available online at: http://www.ameriburn.org/2012NBRAnnualReport.pdf.

2. American Burn Association, National Burn Repository Version 12.0. 2016.

3. Wolf SE, Tompkins RG, Herndon DN. On the Horizon – Research Priorities in Burns for the Next Decade. Surg Clin N Am. 2014;94:917-930.

4. Burd F, Chiu T. Allogenic skin in the treatment of burns. Clin Dermatol. 2005;23:376-387.

5. Atiyeh BS, Gunn SW, Hayek SN. State of the art in burn treatment. World J Surg. 2005;29(2):131-148.

6. Arturson G. Pathophysiology of the burn wound. Ann Chir Gynaecol. 1980;69:178-190.

7. Demling RH. e burn edema process: current concepts. J Burn Care Rehabil. May/June 2005;26:207-227.

8. Boykin JV, Eriksson E, Pittman RN. In vivo microcirculation of a scald burn and the progression of postburn dermal ischemia. Plast Reconstr Surg. 1980;66:191-198.

9. Monafo WW. Initial management of burns. NEJM. 1996; 335(21):1581-1586.

10. Arturson G. e pathophysiology of severe thermal injury. J Burn Care Rehabil. 1985;6(2):129-146.

11. Heggers JP, Robson MC, Zachary LS. romboxane inhibitor for the prevention of progressive dermal ischemia due to the thermal injury. J Burn Care Rehabil. 1985;6:466-468.

12. Miller TA, Korn HN. Epithelial burn injury and repair, In: Davis JC, Hunt TK, editors. Hyperbaric oxygen therapy. Bethesda, MD: Undersea Medical Society, Inc.; 1977. P. 251.

13. Demling RH. Burns and other thermal injuries. In: Way LW, Doherty GM, editors. Current surgical diagnosis and treatment. 11th ed. New York, NY: McGraw-Hill Medical; 2003. P. 267.

14. Alexander JW, Meakins JL. A physiological basis for the development of opportunistic infections in man. Annals of Surgery. 1972;176:273.

15. Alexander JW, Wixson D. Neutrophil dysfunction and sepsis in burn injury. Surg Gynec Obstet. 1970;130:431.

16. Barber RC, Aragaki CC, Rivera-Chavez FA. TLR4 and TNF-alpha polymorphisms are associated with an increased risk for severe sepsis following burn injury. J Med Genet. 2004;41:808-813.

17. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. Clin Microbiol Rev. 2006;19(2):403-434.

18. Deitch EA, Wheelahan TM, Rose MP, Clothier J, Cotter J. Hypertrophic burn scars: Analysis of variables. J Trauma. 1983;23:895-898.

19. Ikeda K, Ajiki H, Nagao H, Karino K, Sugii S, Iwa T, Wada J. Experimental and clinical use of hyperbaric oxygen in burns. In: Wada J, Iwa JT, editors. Proceedings of the fourth international congress on hyperbaric medicine. Baltimore, MD: Williams and Wilkins; 1970. P. 370.

20. Hartwig J, Kirste G. Experimentele untersuchungen uber die revaskularisierung von verbrennungswunden unter hyperbarer sauerstotherapie. Zbl Chir. 1974;99:1112-1117.

21. Nylander G, Nordstrom H, Eriksson E. Eects of hyperbaric oxygen on oedema formation after a scald burn. Burns Incl Therm Inj. 1984 Feb;10(3):193-196.

22. Kaiser W, Schnaidt U, von der Leith H. Auswirkungen hyperbaren sauerstoes auf die fresche brandwunde. Handchir Mikrochir Plast Chir. 1989;21:158-163.

23. Kaiser W, Voss K. Inuence of hyperbaric oxygen on the edema formation in experimental burn injuries. Iugoslaw Physiol Pharmacol Acta. 1992;28(9):87-98.

24. Ketchum SA, Zubrin JR, omas AN, Hall AD. Effect of hyperbaric oxygen on small rst, second and third degree burns. Surg Forum. 1967;18:65-67.

25. Ketchum SA, Thomas AN, Hall AD. Angiographic studies of the effect of hyperbaric oxygen on burn wound revascularization. In: Wada J, Iwa JT, editors. Proceedings of the fourth inter-national congress on hyperbaric medicine. Tokyo: Igaku Shoin Ltd.; 1970. P. 388.

26. Niccole MW, Thornton JW, Danet RT, Bartlett RH, Tavis MJ. Hyperbaric oxygen in burn management: a controlled study. Surgery. 1977;82:727-733.

27. Gruber RP, Brinkley B, Amato JJ, Mendelson JA. Hyperbaric oxygen and pedicle aps, skin grafts, and burns. Plast and Recon Surg. 1970;45:24-30.

28. Wells CH, Hilton JG. Eects of hyperbaric oxygen on post-burn plasma extravasation. In: Hunt TK, Davis JC, editors. Hyperbaric oxygen therapy. Bethesda, MD: Undersea and Hyperbaric Medical Society; 1977. P. 259.

29. Stewart RJ, Yamaguchi KT, Cianci PE, Knost PM, Samadani S, Mason SW, Roshdieh B. Effects of hyperbaric oxygen on adenosine triphosphate in thermally injured skin. Surg Forum. 1988; 39:87.

30. Stewart RJ, Yamaguchi KT, Cianci PE, Mason WW, Roshdieh BB, Dabbassi N. Burn wound levels of ATP after exposure to elevated levels of oxygen. In: Proceedings of the American Burn Association, New Orleans, LA; 1989. P. 67.

31. Germonpré P, Reper P, Vanderkelen A. Hyperbaric oxygen therapy and piracetam decrease the early extension of deep partial thickness burns. Burns. 1996;22(6):468-473.

32. Korn HN, Wheeler ES, Miller TA. Eect of hyperbaric oxygen on second-degree burn wound healing. Arch Surg.1977;112:732-737.

33. Saunders J, Fritz E, Ko F, Bi C, Gottlieb L, Krizek T. e eects of hyperbaric oxygen on dermal ischemia following thermal injury. In: Proceedings of the American Burn Association. New Orleans, LA; 1989. P. 58.

34. Perrins DJD. Failed attempt to limit tissue destruction in scalds of pig's skin with hyperbaric oxygen. In: Wada J, Iwa T, editors. Proceedings of the fourth international congress on hyperbaric medicine. Tokyo, Japan: Igaku Shoin Ltd.; 1970. P. 381.

35. Traystman RJ, Kirsch JR, Koehler RC. Oxygen radical mechanisms of brain injury following ischemia and reperfusion. J Appl Physiol. 1991;71:1185-1195.

36. Ward PA, Mulligan MS. New insights into mechanisms of oxyradical and neutrophil mediated lung injury. Klin Wochenschr. 1991;69:1009-1011.

37. Ward PA, Till GO. e autodestructive consequences of thermal injury. J Burn Care Rehabil. 1985;6:251-255.

38. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med. 1985;312:159-163.

39. Yogaratnam JZ, Laden G, Madden LA, Grin S, et al. Hyperbaric oxygen: a new drug in myocardial revascularization and protection? Cardiovasc Revasc Med. 2006 Jul-Sep;7(3):146-154.

40. Zamboni WA, Roth AC, Russell RC, Graham B, Suchy H, Kucan JO. Morphological analysis of the microcirculation during reperfusion of ischemic skeletal muscle and the effect of hyperbaric oxygen. Plast Reconstr Surg. 1993;91: 1110-1123.

41. Zamboni WA, Stephenson LL, Roth AC, Suchy H, Russell RC. Ischemia-reperfusion injury in skeletal muscle: CD18 dependent neutrophil-endothelial adhesion. Undersea Hyperb Med. 1994; 21(Suppl):53.

42. Wasiak J, Bennett M, Cleland H. Hyperbaric oxygen as adjuvant therapy in the management of burns: can evidence guide clinical practice? Burns. 2006;32:650-652.

43. Buras JA, Stahl GL, Svoboda KK, Weenstra WR. Hyperbaric oxygen down regulates ICAM-1 expression induced by hypoxia and hypoglycemia: the role of NOS. Am J Physiol Cell Physiol. 2000;278:C292-302.

44. Ueno S, Tanabe G, Kihara K et al. Early post-operative hyperbaric oxygen therapy modies neutrophile activation. Hepatogastroenterology. 1999;46:1798-1799.

45. Miljkovic-Lolic M, Silbergleit R, Fiskum G, Rosenthal RE. Neuroprotective eects of hyperbaric oxygen treatment in experimental focal cerebral ischemia are associated with reduced brain leukocyte myeloperoxidase activity. Brain Res. 2003 May 2;971(1):90-94.

46. Shoshani O, Shupak A, Barak Y, Ullman Y, Ramon Y, Lindenbaum E, Peled Y. Hyperbaric oxygen therapy for deep second degree burns: an experimental study in the guinea pig. Brit J Plast Surg. 1998;51:67-73.

47. Bleser F, Benichoux R. Experimental surgery: The treatment of severe burns with hyperbaric oxygen. J Chir (Paris). 1973; 106:281-290.

48. Tenenhaus M, Hansbrough JF, Zapata-Sirvent R, Neumann T. Treatment of burned mice with hyperbaric oxygen reduces mesenteric bacteria but not pulmonary neutrophil deposition. Arch Surg. 1994;129:1338-1342.

49. Magnotti LJ, Deitch EA. Burns, bacterial translocation, gut barrier function, and failure. J of Burn Care Rehab. 2005;26(5): 383-391.

50. Yamada T, Taguchi T, Hirata Y, Suita S, Yugi H. The protective effect of hyperbaric oxygenation on the small intestine in ischemia-reperfusion injury. J Pediatr Surg. 1995;30:786-790.

51. Nylander G, Nordstrom H, Lewis D, Larsson J. Metabolic effects of hyperbaric oxygen in postischemic muscle.Plast Reconstr Surg. 1987;79:91-97.

52. Takahashi M, Iwatsuki N, Ono K, Koga Y. Hyperbaric oxygen therapy accelerates neurologic recovery after 15-minute complete global cerebral ischemia in dogs. Crit Care Med. 1992;20(11): 1588-1594.

53. Thom SR. Functional inhibition of leukocyte B2 integrins by hyperbaric oxygen in carbon monoxide-mediated brain injury in rats. Toxicol Appl Pharmacol. 1993;123:248-256.

54. Veltkamp R, Siebing DA, Schwab S, Schwaninger M. Hyperbaric oxygen reduces blood-brain barrier damage and edema after transient focal cerebral ischemia. Stroke. 2005; 36:1679-1683.

55. Kolski JM, Mazolewski PJ, Stephenson LL, Zamboni WA. Effect of hyperbaric oxygen therapy on testicular ischema-reperfusion injury. J of Urology. Aug 1998;160:601-604.

56. Shandling AH, Ellestad MH, Hart GB, et al. Hyperbaric oxygen and thrombolysis in myocardial infarction: the hot MI pilot study. Am Heart J. 1997;134:544-550.

57. Shari M, Fares W, Abdel-Karim I, Koch JM, Sopko J, Adler D. Hyperbaric oxygen therapy in percutaneous coronary interventions investigators. Usefulness of hyperbaric oxygen therapy to inhibit restenosis after percutaneous coronary intervention for acute myocardial infarction or unstable angina pectoris. Am J Cardiol. 2004 Jun 15;93(12):1533-1535.

58. Thomas MP, Brown LA, Sponseller DR, et al. Myocardial infarct size reduction by synergistic eect of hyperbaric oxygen and recombinant tissue plasminogen activator. Am Heart J. 1990 Oct;120(4):791-800.

59. Xu N, Li Z, Luo X. Effects of hyperbaric oxygen therapy on the changes in serum sIL-2R and Fn in severe burn patients. Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi. 1999;15(3): 220-223.

60. Deitch EA, Xu DZ, Franko L, et al. Evidence favoring the role of the gut as a cytokine generating organ in rats subjected to hemorrhagic shock. Shock. 1994:1:141-146.

61. Deitch EA. Role of the gut lymphatic system in multiple organ failure. Current Opin Crit Care. 2001;7:92-98.

62. Hohn DC, McKay RD, Halliday B, Hunt TK. Effect of oxygen tension on the microbicidal function of leukocytes in wounds and in vitro. Surg Forum. 1976;27:18-20.

63. Allen DB, Maguire JJ, Mahdavian M, et al. Wound hypoxia and acidosis limit neutrophil bacterial killing mechanisms. Arch Surg. 1997;132:991-996.

64. Mader JT, Brown GL, Guckian JC, et al. A mechanism for the amelioration by hyperbaric oxygen of experimental staphylococcal osteomyelitis in rabbits. J Infect Dis. 1980;142:915-922.

65. Hussman J, Hebebrand D, Erdmann D, Moticka J. Lymphocyte subpopulations in spleen and blood after early wound debridement and acute/chronic treatment with hyperbaric oxygen. Hanchir Mikrochir Plast Chir.1996;28(2):103-107.

66. Bilic I, Petri NM, Bota B. Effects of hyperbaric oxygen therapy on experimental burn wound healing in rats: A randomized controlled study. Undersea Hyperb Med. 2005;32(1):1-9.

67. Turkaslan T, Yogum N, Cimsit M, Solakoglu S, Ozdemir C, Ozsoy Z. Is HBOT treatment effective in recovering zone of stasis? An experimental immunohistochemical study. Burns. 2010; 36(4):539-544.

68. Gallagher KA, Goldstein LJ, om SR, Velazquez OC. Hyperbaric oxygen and bone marrow-derived endothelial progenitor cells in diabetic wound healing. Vascular. 2006;14(6):328-337.

69. Gallagher KA, Liu ZJ, Xiao M, Chen H, Goldstein LJ, Buerk DG, Nedeau A, Thom SR, Velazquez OC. Diabetic impairments in NO-mediated endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1 alpha. J Clin Invest. 2007;117:1249-1259.

70. Thom SR, Bhopale VM, Velazquez OC, Goldstein LJ, Thom LH, Buerk DG. Stem cell mobilization by hyperbaric oxygen. Am J Physiol Heart Circ Physiol. 2006;290:H1378-1386.

71. Goldstein LJ, Gallagher KA, Bauer SM et al. Endothelial progenitor cell release into circulation is triggered by hyperoxia-induced increases in bone marrow nitric oxide. Stem Cells. 2006;24:2309-2318.

72. Thom SR, Milovanova TN, Yang M, Bhopale VM, Sorokina EM, Uzun G, Malay DS, Troiano MA, Hardy KR, Lambert DS, Logue CJ, Margolis DJ. Vasculogenic stem cell mobilization and wound recruitment in diabetic patients: increased cell number and intracellular regulatory protein content associated with hyperbaric oxygen therapy. Wound Repair Regen. 2011;19(2): 149-161.

73. Milovanova TN, Bhopale VM, Sorokina EM, Moore JS, Hunt TK, Hauer-Jensen M, Velazquez OC, Thom SR. Hyperbaric oxygen stimulates vasculogenic stem cell growth and dierentiation in vivo. J Appl Physiol. 2009;106:711-728.

74. Milovanova TN, Bhopale VM, Sorokina EM, Moore JS, Hunt TK, Hauer-Jensen M, Velazquez OC, Thom SR. Lactate stimulates vasculogenic stem cells via the thioredoxin system and engages an autocrine activation loop involving hypoxia-inducible factor 1. Mol Cell Biol. 2008;28:6248-6261.

75. Wada J, Ikeda T, Kamata K, Ebuoka M. Oxygen hyperbaric treatment for carbon monoxide poisoning and severe burn in coal mine (hokutanyubari) gas explosion. Igakunoaymi (Japan). 1965;5:53.

76. Ikeda K, Ajiki H, Kamiyama T, Wada J. Clinical application of oxygen hyperbaric treatment. Geka (Japan). 1967;29:1279.

77. Wada J, Ikeda K, Kagaya H, Ajiki H. Oxygen hyperbaric treatment and severe burn. Jap Med J. 1966;13:2203.

78. Lamy ML, Hanquet MM. Application opportunity for OHP in a general hospital - a two year experience with a monoplace hyperbaric oxygen chamber. In: Wada J, Iwa JT, editors. Proceedings of the fourth international congress on hyperbaric medicine. Tokyo: Igaku Shoin Ltd.; 1970. P. 517.

79. Tabor CG. Hyperbaric oxygenation in the treatment of burns of less than forty percent. Korean J Intern Med. 1967;10(4): 267-275.

80. Grossman AR, Grossman AJ. Update on hyperbaric oxygen and treatment of burns. Hyperbaric Oxygen Review. 1982;3:51.

81. Niu AKC, Yang C, Lee HC, Chen SH, Chang LP. Burns treated with adjunctive hyperbaric oxygen therapy: A comparative study in humans. J Hyperbar Med. 1987;2:75.

82. Cianci P, Lueders H, Lee H, Shapiro R, Sexton J, Williams C, Green B. Adjunctive hyperbaric oxygen reduces the need for surgery in 40-80% burns. J Hyperbar Med. 1988;3:97.

83. Cianci P, Lueders HW, Lee H, Shapiro RL, Sexton J, Williams C, Sato R. Adjunctive hyperbaric oxygen therapy reduces length of hospitalization in thermal burns. J Burn Care Rehabil. 1989;10:432-435.

84. Cianci P, Lueders H, Lee H, Shapiro R, Green B, Williams C. Hyperbaric oxygen and burn fluid requirements: Observations in 16 patients with 40-80% TBSA burns. Undersea Biomed Res. 1988;15(Suppl):14.

85. Hart GB, O'Reilly RR, Broussard ND, Cave RH, Goodman DB, Yanda RL. Treatment of burns with hyperbaric oxygen. Surg Gynecol Obstet. 1974 Nov;139(5):693-696.

86. Waisbren BA, Schutz D, Collentine G, Banaszak E. Hyperbaric oxygen in severe burns. Burns. 1982;8:176-179.

87. Merola L, Piscitelli F. Considerations on the use of HBO in the treatment of burns. Ann Med Nav. 1978;83:515.

88. Cianci P, Williams C, Lueders H, Lee H, Shapiro R, Sexton J, Sato R. Adjunctive hyperbaric oxygen in the treatment of thermal burns - an economic analysis. J Burn Care Rehabil. 1990;11: 140-143.

89. Cianci P, Sato R. Adjunctive hyperbaric oxygen therapy in the treatment of thermal burns: A review. Burns. 1994 Feb;20(1): 5-14.

90. Maxwell G, Meites H, Silverstein P. Cost effectiveness of hyperbaric oxygen therapy in burn care. Presented at: Winter Symposium on Baromedicine; 1991; Aspen, CO.

91. Cianci P, Sato R, Green B. Adjunctive hyperbaric oxygen reduces length of hospital stay, surgery, and the cost of care in severe burns. Undersea Biomed Research Suppl. 1991;18:108.

92. Hammarlund C, Svedman C, Svedman P. Hyperbaric oxygen treatment of healthy volunteers with UV-irradiated blister wounds. Bums. 1991;17:296-301.

93. Niezgoda JA, Cianci P, Folden BW, Ortega RL, Slade JB, Storrow AB. The effect of hyperbaric oxygen therapy on a burn wound model in human volunteers. Plast Reconstr Surg. 1997;99(6):1620-1625.

94. Brannen AL, Still J, Haynes M, Orlet H, Rosemblum F, Law E, ompson WO. A randomized prospective trial of hyperbaric oxygen in a referral burn center population. Am Surg. 1997;63:205-208.

95. Sutherland AM, Clarke HA, Katz J, Katznelson R. Hyperbaric oxygen therapy: a New treatment for chronic pain? Pain Practice. 2015;2(1):1-9.

96. Ramussen VM, Borgen AE, Jansen EC, Rotboll Nielsen PH, Werner MU. Hyperbaric oxygen therapy attenuates central sensitization induced by a thermal injury in humans. Acta Anaesthesiologica Scandinavica. 2015;59:749-762.

97. Chong SJ, Kan EM, Song C, Soh CR, Lu J. Work in progress – characterization of early thermal burns and the effects of hyperbaric oxygen treatment: a pilot study. Diving Hyperb Med. 2013;43(3):157-161.

98. Jones LM, Rubadue C, Brown NV, Khandelwal S, Coey RA. Evaluation of TCOM/HBOT practice guideline for the treatment of foot burns occurring in diabetic patients. Burns. 2015;41: 536-541.

99. Zhang Q, Chang Q, Cox RA, Gong X, Gould LJ. Hyperbaric oxygen attenuates apoptosis and decreases inflammation in an ischemic wound model. J Invest Dermatol. 2008;128(8):2102-2112.

100. Fosen KM, om SR. Hyperbaric oxygen, vasculogenic stem cells, and wound healing. Antioxidants & Redox Signaling. 2014; 21(11):1634-1646.

101. Thom SR, Bhopale VM, Velazquez OC, Goldstein LJ, Thom LH, Buerk DG. Stem cell mobilization by hyperbaric oxygen. Am J Physiol Heart Circ. Physiol. 2006;290:H1378-H1386.

102. Thom SR, Milovanova TN, Yang M, Bhopale VM, Sorokina EM, Uzun G, Malay DS, Troiano MA, Hardy KR, Lambert DW, Logue CJ, Margolis DJ. Vasculogenic stem cell mobilization and wound recruitment in diabetic patients: increased cell number and intracellular protein content associated with hyperbaric oxygen therapy. Wound Rep Reg. 2011;19:149-161.

103. Stewart RJ, Yamaguchi KT, Cianci PE, Knost PM, Samadani S. Mason SW, Roshdieh B. Effects of hyperbaric oxygen on adenosine triphosphate in thermally injured skin. Surg Forum. 1988;39:87.

104. Stewart RJ, Yamaguchi KT, Cianci PE, Mason WW, Roshdieh BB, Dabbassi N. Burn wound levels of ATP after exposure to elevated levels of oxygen. Proceedings of the American Burn Association, New Orleans. 1989:67.

105. Sureda A, Batle JM, Martorell M, Capo X, Tejada S, Tur JA, Pons A. Antioxidant response of chronic wounds to hyperbaric oxygen therapy. PLoS One 2016;11(9):e0163371.

106. Shirani K, Pruitt B, Mason A. The influence of inhalation injury and pneumonia on burn mortality. Ann Surg.1986;205: 82-87.

107. Balkissoon R, Shusterman DJ. Occupational upper airway disorders. Semin Respir Crit Care Med. 1999;20:569-580.

108. Rabinowitz, PM, Siegel MD. Acute inhalation injury. Clin Chest Med. 2002;23(4):707.

109. Grim PS, Nahum A, Gottlieb L, Wilbert C, Hawe E, Sznajder J. Lack of measurable oxidative stress during HBO therapy in burn patients. Undersea Biomed Res. 1989;16(Suppl):22.

110. Ray CS, Green G, Cianci P. Hyperbaric oxygen therapy in burn patients: Cost effective adjuvant therapy (abstract). Undersea Biomed Res. 1991;18(Suppl):77.

111. Villanueva E, Bennett MH, Wasiak J, Lehm JP. Hyperbaric oxygen therapy for thermal burns (Review). Cochrane Database Syst Rev. 2004;(3):CD004727.

112. Hunt JL, Sato RM, Baxter CR. Early tangential excision and immediate mesh auto-grafting of deep dermal hand burns. Annals Surg. 1979;189(2):147-151. (Orig paper)

113. Sato RM, Beesinger DE, Hunt JL, Baxter CR. Early excision and closure of the burn wound. Current Topics in Burn Care. TL Wachtel et al.(eds) Rockville, Aspen Publication;1983. 65-76.(Orig paper)

114. Sato RM, Baxter CT. Tangential excision of the burn wound. Recent advances in emergency and definitive burn wound care. Proceedings of a Symposium Sponsored by Valley Medical Center (Fresno, CA). March 1977:16-24. CV2.

115. Sato R, Beesinger D, Hunt J, Baxter C. Early excision and closure of the burn wound. Critical Care Quarterly. 1978;1(3): 51-62. CV4.

116. Hunt JL, Sato RM. Acute electrical burns. uncommon problems in emergency medicine. Michael I. Greenberg (ed). Philadelphia, F.A. Davis Company; 1982. Pp:183-195. CV11.

117. Hunt JL, Sato RM. Early excision of full thickness hand and digit burns: factors affecting morbidity. J Trauma. 1982;22(5): 414-419. CV12.

118. Kowalczyk L. Catastrophic costs: hospitals, insurers, some R.I. fire victims face huge medical bills. The Boston Globe. 2003 Feb 28.

119. Hunt JL, Sato RM, Baxter CR. Early tangential excision and immediate mesh auto-grafting of deep dermal hand burns. Annals Surg. 1979;189(2):147-151.

120. Sato RM, Beesinger DE, Hunt JL, Baxter CR. Early excision and closure of the burn wound. In: Wachtel TL et al., editors. Current topics in burn care. Rockville, MD: Aspen Publications; 1983. 65-76.

121. Nichter LS, Morwood DT, Williams GS, Spence RJ. Expanding the limits of composite grafting: A case report of successful nose replantation assisted by hyperbaric oxygen therapy. Plast Reconstr Surg. 1991;87:337-340.

122. Kindwall EP. e use of drugs under pressure. In: Kindwall EP, Whelan HT, editors. Hyperbaric medicine practice. 2nd ed. Flagsta, AZ: Best Publishing Co.; 1999. P. 326.

123. Personal experience of the authors in a regional burn center.

124. Grube BJ, Marvin JA, Heimbach DM. Therapeutic hyperbaric oxygen: Help or hindrance in burn patients with carbon monoxide poisoning? J Burn Care Rehabil. 1988;9.

125. Cost statistics (1997-98) from hospital patient accounts, home facility of the authors.

126. Ong YS, Samual M, Song C. Meta-analysis of early excision of burns. Burns. 2006;32(2):145-150.

127. Engrav LH, Heimbach DM, Rivara FP et al. Harborview burns 1974-2009. PlosOne. 2012;7(7):1-23.

128. Blaisdell LL, Chace R, Hallagan LD, Clark DE. A half century of burn epidemiology and burn care in a rural state. J Burn Care Res. 2012 May-Jun;33(3):347-353.

129. Rowan MP, Cancio LC, Elster EA, Burmeister DM, Rose LF, Natesan S, Chan RK, Christy RJ, Chung KK. Burn wound healing and treatment: review and advancements. Critical Care. 2015; DOI 10.1186/s13054-01509861-2.

130. Boykin JV Jr. Letter to the Editor, Undersea Hyperb Med. 2013 Mar-Apr;40(2):212.