Wound oxygen levels during hyperbaric oxygen treatment in healing wounds.

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Rollins M.D., Gibson J.J., Hunt T.K., Hopf H.W. Wound oxygen levels during hyperbaric oxygen treatment in healing wounds. Undersea Hyperb Med 2006; 33(1):17-25.Hyperbaric oxygen (HBO_2) increases wound oxygen delivery, but few data quantify wound oxygen levels over the course of healing. We characterized these changes during and after HBO_2 treatment in a rat wound model. The treatment group (n=7) received 2.0 ATA HBO_2, 90 minutes BID for 15 days. Control rats (n=5) were only exposed to HBO_2 during measurement. On days 5, 10, and 15, wound pO_2 was measured before, during, and for an hour after HBO_2 treatment. Both the peak pO_2 and the pO_2 one hour after HBO_2 treatment were significantly greater than baseline on all days in both the treatment ($p < .01$) and control group ($p < .05$). The peak pO_2 during HBO_2 exposure and one hour after decreased significantly in the treatment group on day 15 compared to day 5 ($p <.01$, $p <.05$ respectively). No significant differences were found in pO_2 values between days within the control group. These results demonstrate that both the peak wound oxygen levels and duration of elevation change significantly throughout the course of HBO_2 treatment.

INTRODUCTION

Oxygen is critical to wound healing, and an abundant supply is required and central to numerous healing processes. The partial pressure of oxygen (pO_2) is positively correlated with angiogenesis (1-3), collagen deposition (4, 5), epithelization (6, 7), and superoxide production (8-12) for intracellular bacterial killing. Oxygen delivery to wounds is complex and depends upon the interaction of blood perfusion, arterial pO_2, intercapillary density and spatial arrangement, oxyhemoglobin dissociation conditions, mass transfer resistances, and local oxygen consumption rate. Oxygen diffuses along a partial pressure gradient, and delivery is inversely proportional to the square of the diffusion distance. Because of the extended intercapillary distance, pO_2 is often nearly zero in the central devascularized region of a wound. Transport of oxygen to the hypoxic center of a wound, or to a wound with impaired perfusion, is often only possible with hyperbaric oxygen (HBO_2) therapy (13-15). Several studies have shown that HBO_2 promotes wound healing, especially in hypoxic wounds (14-19).

Because of the numerous variables affecting wound pO_2, accurate determination of wound pO_2 during HBO_2 treatment requires direct measurement. The tissue changes occurring during the wound healing process may affect the wound pO_2 attained during HBO_2 treatment. An objective, quantitative measure of wound oxygen tension throughout and after a single HBO_2 treatment, measured on specific days during the course of healing is necessary to better understand the effects and mechanisms of HBO_2 therapy. In previous studies, human wound and subcutaneous tissue pO_2 levels increased markedly during a single

HBO_2 treatment (16, 20-23). The degree of this increase above the pO_2 measured under room air conditions showed a high degree of variability between individuals, with both increases and decreases in the peak pO_2 attained over weeks of HBO_2 treatment in individual case study patients (20, 21, 24). Given the high degree of variability seen in the human case studies, we opted to measure the specific changes in oxygen delivery during a course of HBO_2 therapy using a well characterized animal wound model under controlled conditions.

The Hunt-Schilling dead space wound model has been widely used to evaluate the effects of oxygen on wound healing (25). In this model, HBO_2 treatment increases wound fluid VEGF levels (26), and improves bacterial killing (27). Wound fluid pO_2 in this model was previously measured in a normobaric hyperoxic environment (28) and during bacterial infection (29, 30).

The goal of this study was to characterize the response in dead space wound pO_2 before, during, and after a single HBO_2 treatment on specific days in rats receiving daily serial HBO_2 treatments, as well as in those not receiving daily HBO_2 treatments. This information should further the understanding of HBO_2 delivery and the impact of serial wound treatments.

METHODS

All animal experimentation was performed with approval of the University of California, San Francisco Committee on Animal Research, using established guidelines.

Wound model

A wire mesh wound cylinder was implanted subcutaneously in 18 female Sprague-Dawley (250-300 gram) rats (Day 0) using techniques similar to those previously described (25, 26, 30, 31). The cylinders were made by cutting 28 mm by 33 mm rectangles from stainless steel wire screen (#316 steel, #40 mesh, 0.01 diameter wire, Cambridge Wire Cloth Co., Cambridge, MD). The rectangles were rolled into cylinders with a length of 28 mm. The ends were inserted into the caps from two 2 mL cryovials in order to maintain the cylindrical form (Nalge Nunc International, Rochester, NY). One end cap was removed and filled to half of its total volume with a medical grade silicone elastomer (MDX4-4210 Factor II, Inc., Lakeside, AZ) mixed with a curing agent. The cap was replaced and the assembly centrifuged at 1000 X g for 10 minutes in order to force the elastomer into the screen spacing and remove any air bubbles. The end was polymerized by heating for 30 minutes at 75°C. The process was then repeated for the other end, and the cryovial caps removed. The final product was a stainless steel wire mesh cylinder, 1.0 cm. in diameter and 3.0 cm. long, with 3 mm. thick silicone endcaps. The cylinders were then heat sterilized prior to implantation.

Under sterile conditions and halothane anesthesia, a 2.5 cm. midline skin incision was created on the dorsum of each rat. A subcutaneous space was created with blunt dissection and the cylinder was implanted into the space to lay 1.5 cm lateral to midline with its axis parallel to the spine. The wound was closed with 3-4 skin staples. These were removed on postoperative day 10.

HBO_2 treatment and monitoring

Two groups of rats were studied: The HBO_2 treatment group received daily HBO_2 treatments (treatment group, n=10) while the control group was exposed to HBO_2 only during measurement periods (control group, n=8). HBO_2 treatment rats were exposed (starting on Day 1) to 100% O_2 at 2.0 ATA for 90 minutes twice a day for 15 days, while control rats(n = 8) were maintained in room air and received a single treatment of 100% O_2 at 2.0 ATA

for 90 minutes on days 5, 10, and 15, during measurement.

On postoperative days 5, 10, and 15, the pO_2 inside the wound cylinder was measured before and during HBO_2 treatment and for one hour after its completion. A LICOX polarographic oxygen and temperature probe system (Medical Systems Corp., Greenvale, NY) was used to measure the oxygen concentration. The electrode used was the "Microcatheter pO_2 probe", Model C1 (OD=0.47mm, Length = 200mm, integrating pO_2 sensitive area = 5mm in length, sensitivity = 2.5x10-9 A / mmHg pO_2, gold cathode polarized to 795 mV). The electrode is housed in a weak polyethelene catheter with a thickness of approximately 70μm. The system assumes a linear relationship between pO_2 and current and is designed for a two point calibration at 0% oxygen and 21% oxygen at 1.0 ATA. Because the wound oxygen tensions were assumed to be out of this range, the accuracy of the measurement system was previously evaluated under conditions of high oxygen concentrations (32). This study showed the need for empiric correction for pO_2 values greater than 400mmHg in order to reduce the measurement error to <5%, and therefore correction was applied to appropriate values obtained during this study. During measurement, the rats were anesthetized with pentobarbital (35 mg/kg) and buprenorphine (0.05 mg/kg) and received atropine (0.8 mg/kg) to counteract anesthetic cardiac and respiratory depression. A 2 mm skin incision was created 1 cm away from each end of the implanted cylinder. An 18 gauge hubless spinal needle was inserted subcutaneously through the first skin nick, piercing both ends of the cylinder and returning through the second skin nick. The oxygen electrode was inserted into the base of the needle, and the needle pulled through the cylinder and skin, leaving the probe inserted through the cylinder with the tip visible outside the rat. The probe was gently pulled back into the cylinder a measured distance, placing the distal measuring portion near the center of the cylinder. This process was repeated for placement of the temperature probe.

After a baseline wound cylinder pO_2 was established (change in $pO_2 \leq 1$ mmHg over 5 minutes with the rat breathing room air), the rat was placed under 100% O_2 at 2.0 ATA for 90 minutes and then returned to room air conditions. Data were acquired and recorded every 15 seconds throughout the experiment using LICOX computer software. Rat subcutaneous temperature was maintained at 35 - 38°C throughout experimentation. An electric heating pad was used outside the chamber, and warmed saline-filled bags were used within the HBO_2 chamber. Rats were given 50% of the initial anesthetic dose immediately after removal from the HBO_2 chamber to maintain adequate anesthetic depth for the remainder of the measurement. After completion of the in vivo pO_2 measurement, the position of the distal probe tip was confirmed, and the polarographic electrode was returned to room air conditions to determine if any drift had occurred. Data were not used if the measuring electrode was determined to be in the silicone ends of the cylinder, or if the probe did not return to within 10% of its initial room air calibration value. Due to anesthetic complications, inaccurate probe placement, and probe drift, all three time points were not obtained from every rat. A total of 7 measurements were obtained for each time point in the treatment group and a total of 5 measurements were obtained for each time point in the control group.

To evaluate the possible transfer of oxygen through the skin or probe insertion site into the cylinder, the pO_2 inside the wound cylinder was measured in euthanized rats (n=3) during 45 minutes of HBO_2 exposure. Following completion of the day 15 measurement, the rats were euthanized with CO_2 and bilateral thoracotomies were

performed. The pO_2 electrode measurement system was left in place, and the euthanized rat was placed within the chamber under 100% oxygen at 2 ATA for one hour. Data were acquired and recorded every 15 seconds using LICOX computer software.

Statistical analysis

The pO_2 measured prior to HBO_2 treatment, the peak pO_2 attained during HBO_2 treatment, and the pO_2 1 hour after HBO_2 exposure were compared between days 5, 10, and 15 (ANOVA with Scheffe's post hoc test) in both groups. On each measured day, the pO_2 measured prior to HBO_2 treatment was compared to both the peak pO_2 attained during HBO_2 treatment, and the pO_2 1 hour after HBO_2 exposure using a Student's two tailed t-test. In addition, the increases in wound oxygen content above baseline during and after treatment (represented by area under the curve in the pO_2 vs. Time plot) were also compared between days 5, 10, and 15 (ANOVA with Scheffe's post hoc test) in both groups. A Student's two tailed t-test was used to compare the two groups for the same measurements on each of the three days measured.

RESULTS

The average pO_2 values for the HBO_2 treatment group (n = 7) and the control group (n = 5) are shown in Figures 1 and 2 respectively. Tables 1 and 2 display the summary of pO_2 analysis for both the treatment and control groups. The increase in wound oxygen exposure is represented by area under the pO_2 vs. time curve.

On all days measured, the peak pO_2 at 90 minutes of HBO_2 treatment was significantly greater than the pO_2 measured prior to beginning HBO_2 in both the control ($p < .05$ on all days) and treatment group ($p < .01$ on all days). On all days measured, the pO_2 one hour after completion of HBO_2 exposure was significantly greater than the pO_2 measured prior to beginning HBO_2 in both the control ($p < .05$ on all days) and treatment group ($p < .01$ on all days).

The pO_2 measured prior to HBO_2 treatment was not significantly different across days in either the control or treatment group. The peak pO_2 attained during HBO_2 treatment, and the pO_2 1 hour after HBO_2 exposure decreased significantly in the HBO_2 treatment group on day 15 compared to day 5 ($p \leq .01$, $p \leq .05$ respectively). No significant differences were found in pO_2 values within the control group across the three days measured. Analysis between groups demonstrated the treatment group attained a significantly higher peak pO_2 on day 5 as compared to control ($p \leq .05$).

The area under the curves in Figures 1 and 2 decreased significantly from day 5 to day 15 in the treatment group during the HBO_2 treatment period, the one hour period after HBO_2 exposure, and over the entire measurement period ($p \leq .001$ in all cases). The area under the curves showed no significant difference between days in the control group.

Analysis between groups demonstrated the treatment group attained a significantly greater area under the curve on day 5 as compared to control during the HBO_2 treatment period, the one hour period after HBO_2 exposure, and the entire measurement period ($p \leq .01$ in all cases).

After euthanasia, the wound cylinder pO_2 in the three rats measured was 10.8, 13.1, and 0.8 mmHg. After a 45 minute exposure to 2 ATA 100% oxygen, the changes in pO_2 were +11.0, -11.5, and -0.3 mmHg respectively.

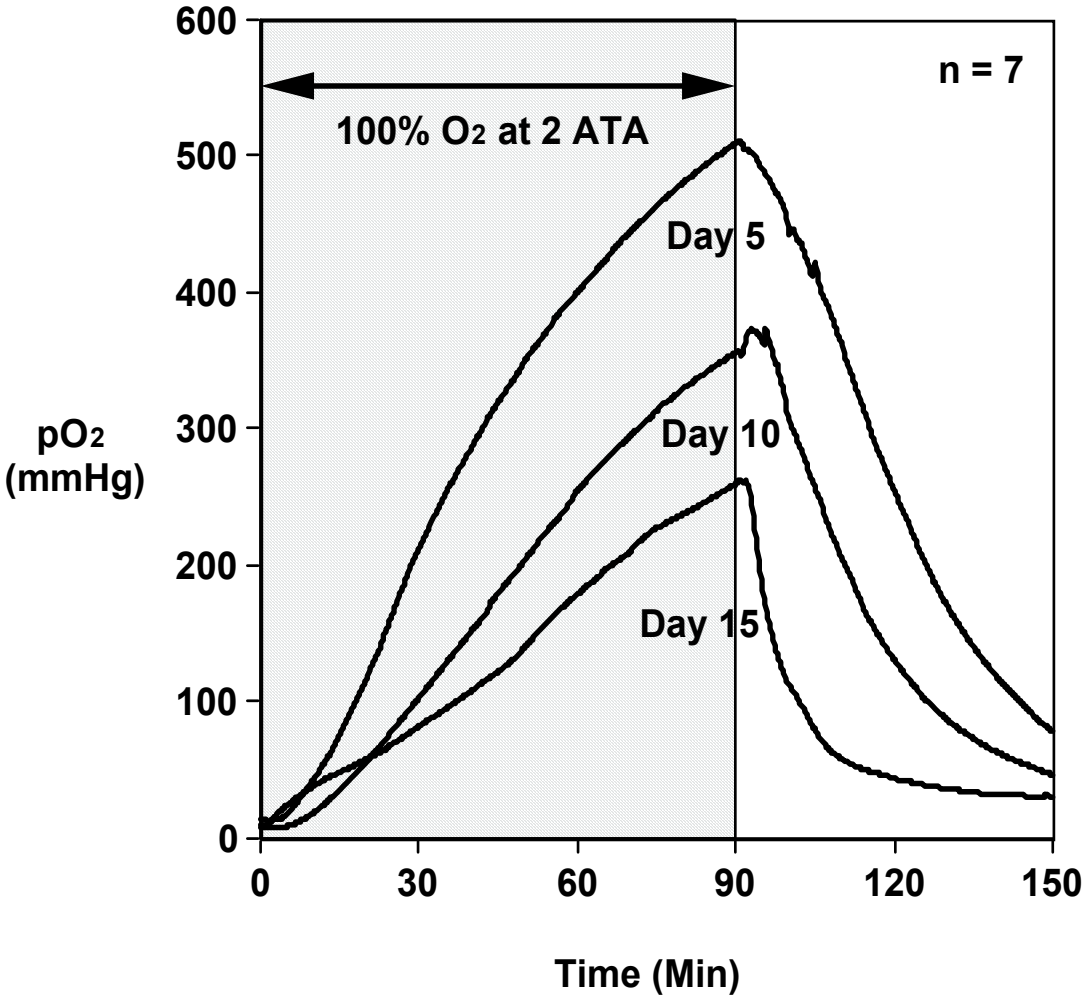

Fig. 1 (Treatment Group): The mean pO_2 values before, during, and up to one hour after HBO_2 exposure for the treatment group are plotted vs. time. Plots are shown for days 5, 10, and 15. The shaded region represents the period of HBO_2 exposure

Table 1 (Treatment Group Results):

	pO_2 values (mmHg)			Area Under Curve (mmHg X min) $X10^{-3}$		
Day	**Start**	**Peak**	**1hr post HBO_2**	**0-90 Min**	**90-150 Min**	**0-150 Min**
5	13 ± 14	541 ± 181**	78 ± 45	24.7 ± 7.5**	15.7 ± 7.1**	40.5 ± 12.9**
10	7 ± 4	392 ± 135	47 ± 15	15.3 ± 4.6*	9.7 ± 4.4*	24.9 ± 8.5
15	9 ± 4	266 ± 124*	31 ± 12*	11.1 ± 5.2*	3.7 ± 0.9*	14.8 ± 5.9*

The mean measured pO_2 values (n=7) at the start, peak, and one hour after exposure are displayed. The mean calculated area under the pO_2 vs time curves during the 90 minute HBO_2 treatment, the 60 minutes post treatment, and the entire 150 minutes measured are also tabulated. The statistical error displayed is standard deviation.

* Statistically significant decrease as compared to day 5 ($p < .05$ in all cases)

** Statistically significant difference as compared to control group ($p < .05$ in all cases)

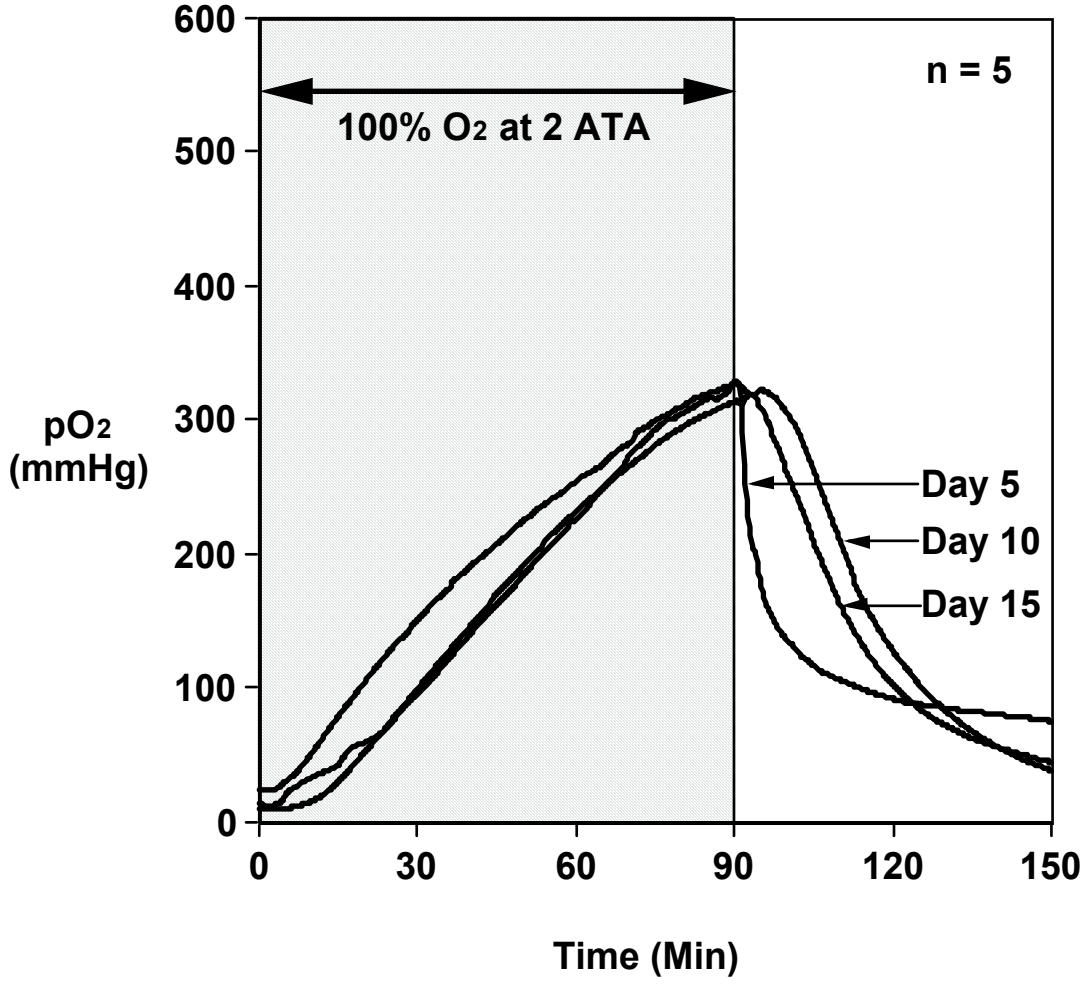

Fig. 2. (Control Group): The mean pO_2 values before, during, and up to one hour after HBO_2 exposure for the control group are plotted vs. time. Plots are shown for days 5, 10, and 15. The shaded region represents the period of HBO_2 exposure.

Table 2 (Control Group Results):

	pO_2 values (mmHg)			Area Under Curve (mmHg X min) $X10^{-3}$		
Day	**Start**	**Peak**	**1hr post HBO_2**	**0-90 Min**	**90-150 Min**	**0-150 Min**
5	14 ± 10	334 ± 123	76 ± 33	13.6 ± 4.6	5.8 ± 1.3	19.5 ± 4.2
10	10 ± 12	323 ± 201	39 ± 30	13.7 ± 9.6	9.0 ± 6.6	22.7 ± 16.1
15	24 ± 22	333 ± 174	45 ± 11	15.3 ± 10.8	7.0 ± 4.5	22.2 ± 15.2

The mean measured pO_2 values (n=5) at the start, peak, and one hour after exposure are displayed. The mean calculated area under the pO_2 vs time curves during the 90 minute HBO_2 treatment, the 60 minutes post treatment, and the entire 150 minutes measured are also tabulated. The statistical error displayed is standard deviation.

DISCUSSION

HBO_2 exposure significantly increases pO_2 in a dead space wound model. Oxygen tension in the rat dead space wound model increased significantly above baseline room air levels during a 90 minute HBO_2 treatment on days 5, 10, and 15 in both the control and treatment groups. The pO_2 remained significantly elevated above baseline for more than an hour after treatment ended in all cases. This finding contrasts to the rapid decrease seen in subcutaneous tissue after completion of HBO_2 exposure (21, 33). The dead space wound model has a large distance (cylinder radius = 0.5 cm) between the center of the dead space and the vascular tissue surrounding the chamber. This creates a large diffusion distance and allows the oxygen to be retained within the wound for a much longer time than would be expected in subcutaneous tissue where the distance between vessels is not as extreme. Additionally, based on the shapes of the curves in Figures 1 and 2, the wound oxygen tension attained at 90 minutes is not a plateau value. Due to the large diffusion distance, the final wound oxygen plateau value under continuous HBO_2 exposure would likely to be significantly greater. We chose a 90 minute treatment period in order to mimic clinical practice and limit oxygen toxicity.

The oxygen tension in the dead space wound represents a dynamic equilibrium between the amount of oxygen delivered, the amount of oxygen removed, and the amount of oxygen consumed. The shape and area under the curve of the pO_2 vs time plot during and after an HBO_2 treatment are determined by all of these factors. The oxygen tension at any given time does not represent oxygen content, but rather a concentration, which is responsible for many biological reactions critical to wound healing such as collagen deposition, angiogenesis, and bacterial killing. The area under the curve of the pO_2 vs time plot represents the dynamic changes occurring between oxygen delivery, removal, and consumption over time, and represents the quantitative sum of these dynamics over a given time.

Over the course of HBO_2 treatment there was a significant decrease in the peak pO_2 at the end of a 90 minute HBO_2 treatment, the area under the curve of pO_2 measured throughout the treatment period, the area under the curve during the hour after the HBO_2 treatment, and the total area from the sum of these two time periods. These results show a decrease in wound oxygen level exposure with successive treatments. A similar finding was demonstrated in a study by Siddiqui et al., measuring the subcutaneous tissue pO_2 in an ischemic rabbit ear model during HBO_2 therapy. The results showed the duration of wound tissue pO_2 elevation above baseline in response to HBO_2 exposure decreased as HBO_2 therapy progressed (33). The degree of sustained wound oxygen elevation appear greater in our study compared with Siddiqui et al., and are likely a function of the increased diffusion distances in our wound model as compared to surrounding the probe with subcutaneous tissue.

The peak pO_2 at 90 minutes of treatment and the area under the curve remained similar across days in the control group. Significant differences between the treatment and control groups were evident on the day 5 measurements, with greater peak pO_2 in the HBO_2 treatment group that disappeared on days 10 and 15. The differences seen between the groups, and across days in the treatment group illustrate changes in not only oxygen delivery, but also removal and consumption, and may represent combined effects from angiogenesis, changes in capsular thickness, size of wound dead space, and total cellularity. Although the specific contribution of each is impossible to discern in our model, the net change in wound oxygenation from HBO_2 exposure is evident.

Among many possible explanations, one plausible mechanism for the net change is the improvement in the wound tissue utilization of the available oxygen during the HBO_2 exposure. Uninfected human dermis consumes approximately 0.7 ml. O2 / 100 ml. blood delivered (34). This number is likely to change in a wound as cellularity increases and superoxide is produced to resist infection. In addition, as oxygen supply to a wound is enhanced, consumption increases (35). HBO_2 is known to increase fibroblast collagen production, fibroblast migration, and capillary budding, and has been shown to increase vessel density in other wound models (36, 37). Future studies are needed to ascertain the role of angiogenesis and increased cellularity in the temporal changes seen in response to HBO_2 therapy.

The lack of change in oxygen tension profiles during HBO_2 exposure in the control group may be due in part to slower healing, since pO_2 levels less than 25 – 40 mmHg are associated with poor healing (5). Wound pO_2 levels were probably well below this level during the vast majority of the study, since the wound model pO_2 under room air conditions is usually < 20 mmHg, as shown by the measurements taken prior to HBO_2 treatment.

Only a negligible amount of oxygen enters the wound through the skin or probe insertion site, as determined from the studies of euthanized rats. This finding demonstrates that the effect of HBO_2 on wound pO_2 results from systemic rather than topical oxygen delivery. This finding reinforces the importance of angiogenesis and blood-borne oxygen delivery under both normobaric and hyperbaric conditions.

HBO_2 treatment greatly increases wound pO_2 even in a dead space wound model, a condition which represents an extreme case of the increased intercapillary distance seen in wounds. This finding supports the effectiveness of HBO_2 in oxygen delivery to hypoxic wounds. The temporal changes seen with daily HBO_2 therapy suggest dynamic biological interactions affecting the degree of wound pO_2 increase during and after HBO_2 treatment. One plausible explanation is that the wound healing response during a course of HBO_2 therapy is enhanced compared to similar wounds not exposed to HBO_2 therapy. This enhancement would increase wound oxygen utilization and would result in the decrease in both peak pO_2 and area under the pO_2 vs time curves seen in this study's treatment group. Future studies are needed to ascertain specific differences occurring in the wound during a course of HBO_2 treatment.

ACKNOWLEDGMENTS

This study was supported by NIH GM 27345 and the Whitaker Foundation

REFERENCES

1. Knighton DR, Hunt TK, Scheuenstuhl H, Halliday BJ, Werb Z, Banda MJ. Oxygen tension regulates the expression of angiogenesis factor by macrophages. *Science* 1983; 221(4617):1283-5.
2. Knighton DR, Silver IA, Hunt TK. Regulation of wound-healing angiogenesis-effect of oxygen gradients and inspired oxygen concentration. *Surgery* 1981; 90(2):262-70.
3. Hopf HW, Gibson JJ, Angeles AP et al. Hypoxia and Angiogenesis. Wound Repair and Regeneration 2005; In Press.
4. Jonsson K, Jensen JA, Goodson WHd et al. Tissue oxygenation, anemia, and perfusion in relation to wound healing in surgical patients. *Annals of Surgery* 1991; 214(5):605-13.
5. Warriner RA, Hopf HW. Enhancement of healing in selected problem wounds. In: Feldmeier JJ, ed. Hyperbaric Oxygen 2003: Indications and Results: The Hyperbaric Oxygen Therapy Committee Report. Kensington, MD: Undersea and Hyperbaric Medical Society, Inc., 2003:42.
6. Bullough W, Johnson M. Epidermal mitotic activity and oxygen tension. *Nature* 1957; 167:488.
7. Uhl E, Sirsjo A, Haapaniemi T, Nilsson G, Nylander G. Hyperbaric oxygen improves wound healing in normal and ischemic skin tissue. *Plast Reconstr Surg* 1994; 93(4):835-41.

8. Chang N, Mathes SJ. Comparison of the effect of bacterial inoculation in musculocutaneous and random-pattern flaps. *Plastic and Reconstructive Surgery* 1982; 70(1):1-10.
9. Hohn DC, MacKay RD, Halliday B, Hunt TK. Effect of O2 tension on microbicidal function of leukocytes in wounds and in vitro. *Surgical Forum* 1976; 27(62):18-20.
10. Jonsson K, Hunt TK, Mathes SJ. Oxygen as an isolated variable influences resistance to infection. *Ann Surg* 1988; 208: 783-7.
11. Knighton DR, Halliday B, Hunt TK. Oxygen as an antibiotic. The effect of inspired oxygen on infection. *Arch Surg* 1984; 119(2):199-204.
12. Allen D, Maguire J, Mahdavian M et al. Wound hypoxia and acidosis limit neutrophil bacterial killing mechanisms. *Archives of Surgery* 1997; 132: 991-6.
13. Silver I. The measurement of oxygen tension in healing tissue. Progress in Respiration Research 1969; 3: 124.
14. Davis JC. The use of adjuvant hyperbaric oxygen in treatment of the diabetic foot. *Clin Podiatr Med Surg* 1987; 4(2):429-37.
15. Faglia E, Favales F, Aldeghi A et al. Adjunctive systemic hyperbaric oxygen therapy in treatment of severe prevalently ischemic diabetic foot ulcer. A randomized study. *Diabetes Care* 1996; 19(12):1338-43.
16. Cianci P, Hunt T. Adjunctive hyperbaric oxygen therapy in the treatment of diabetic wounds of the foot. In: ME Levin; LW O'Neal; JH Bowker, editors, translator and editor The Diabetic foot. 5th edn. St. Louis: Mosby Year Book; 1993.
17. Niinikoski J. Oxygen and wound healing. *Clin Plast Surg* 1977; 4(3):361-74.
18. Uhl E, Sirsjö A, Haapaniemi T, Nilsson G, Nylander G. Hyperbaric oxygen improves wound healing in normal and ischemic skin tissue. *Plastic and Reconstructive Surgery* 1994; 93(4):835-41.
19. Smith BM, Desvigne LD, Slade JB, Dooley JW, Warren DC. Transcutaneous oxygen measurements predict healing of leg wounds with hyperbaric therapy. *Wound Repair and Regeneration* 1996; 4(2):224-9.
20. Sheffield PJ. Tissue Oxygen Measurements with Respect to Soft-Tissue Wound Healing with Normobaric and Hyperbaric Oxygen. *Hyperbaric Oxygen Review* 1985; 6(1):18-31.
21. Sheffield PJ. Tissue Oxygen Measurements. In: JC Davis; TK Hunt, editors, translator and editor Problem Wounds: The Role of Oxygen: New York: Elsevier; 1988; p. 17-51.
22. Wells CH, Goodpasture JE, J. HD, Hart GB. Tissue gas measurements during hyperbaric oxygen exposure. In: G Smith, editor, translator and editor Proceedings of the Sixth International Congress on Hyperbaric Medicine. Aberdeen, Scotland: Aberdeen University Press; 1977; p. 118-24.
23. Sheffield PJ, Dunn JM. Continuous monitoring of tissue oxygen tension during hyperbaric oxygen therapy - a preliminary report. In: G Smith, editor, translator and editor Proceedings of the Sixth International Congress on Hyperbaric Medicine. Aberdeen, Scotland: Aberdeen University Press; 1977; p. 125-9.
24. Wattel F, Mathieu D, Coget JM, Billard V. Hyperbaric oxygen therapy in chronic vascular wound management. *Angiology* 1990; 41(1):59-65.
25. Schilling J, Favata B, Radakovick M. Studies of fibroblast in wound healing. *Surg Gynecol Obstet* 1953; 96:143-49.
26. Gibson JJ, Sheikh AY, Rollins MD, Hopf HW, Hunt TK. Increased Oxygen Tension and Wound Fluid Vascular Endothelial Growth Factor Levels. *Surgical Forum* 1998; 49: 607-10.
27. Hunt TK, Linsey M, Grislis H, Sonne M, Jawetz E. The effect of differing ambient oxygen tensions on wound infection. *Ann Surg* 1975; 181(1):35-9.
28. Niinikoski J, Hunt TK, Dunphy JE. Oxygen supply in healing tissue. *Am J Surg* 1972; 123(3):247-52.
29. Niinikoski J, Grislis G, Hunt TK. Respiratory gas tensions and collagen in infected wounds. *Ann Surg* 1972; 175(4):588-93.
30. Hunt TK, Twomey P, Zederfeldt B, Dunphy JE. Respiratory gas tensions and pH in healing wounds. *Am J Surg* 1967; 114(2):302-7.
31. Schilling JA, Joel W, Shurley HW. Wound healing: a comparative study of the histchemical changes in granulation tissue contained in stainless steel wire mesh and polyvinyl sponge cylinder. *Surgery* 1959; 46: 702.
32. Rollins MD, Conrad MB, Hunt TK, Hopf HW. Accuracy of a polarographic electrode at high oxygen concentrations. *Adv Exp Med Biol* 2003; 510: 169-73.
33. Siddiqui A, Davidson JD, Mustoe TA. Ischemic tissue oxygen capacitance after hyperbaric oxygen therapy: a new physiologic concept. *Plastic and Reconstructive Surgery* 1997; 99(1):148-55.
34. Evans NTS, Naylor PFD. Steady states of oxygen tension in human dermis. *Respiration Physiology* 1966; 2: 46-60.
35. Remensnyder JP, Majno G. Oxygen gradients in healing wounds. *Am J Patho*l 1968; 52(2):301-23.
36. Gibson JJ, Angeles AP, Hunt TK. Increased Oxygen Tension Potentiates Angiogenesis. *Surgical Forum* 1997; 48: 696-9.
37. Hunt TK, Van Winkle WJ. Wound Healing: Normal Repair. In: JE Dunphy, editor, translator and editor Fundamentals of Wound Management in Surgery. South Plainfield, NJ: Chirurgecorn, Inc.; 1976; p. 1-68.